

THE EFFECT OF IRON AND FOLIC ACID
IN
NORMAL PREGNANCY

A Thesis submitted to the University of Wales
to be considered for the degree of Doctor of Medicine

by

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I declare that the work reported in this thesis is my own and has been written by myself except as indicated in the acknowledgements at the end of the preface.

This thesis has not already been accepted for any other degree and is not concurrently being submitted in candidature for any other degree.

Two papers have already been published in collaboration with another describing methods devised for use in this work. Reprints are to be found in the pocket inside the back cover of Volume 2.

Signed_

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PREFACE

There are still differences of opinion as to whether changes in the blood in otherwise normal pregnant women are the result of physiological adaptation to pregnancy or a reflection of sub-clinical deficiencies of iron and folic acid.

In some areas the prevalence of overt anaemia due to deficiency of iron or folic acid is high. It has been demonstrated that these conditions can be almost completely abolished by routine administration of iron and folic acid to all pregnant women. All women, however, do not become clinically anaemic in pregnancy. It is therefore reasonable to suppose that when routine supplements are given a number of pregnant women will receive iron and/or folic acid in excess of the normal requirements. There is little detailed information in the literature on the behaviour of iron and folic acid metabolism in such women.

This project was constructed to follow changes in different variables of the blood which are related to the status of iron and folic acid nutrition in pregnancy. The women studied are all apparently normal, that is they have no obvious deficiencies although the presence of sub-clinical deficiency may be deduced in the final analysis. Comparisons are made between women who are able to remain untreated throughout pregnancy, women who eventually required treatment, women on iron and women on iron + folic acid. Individual results are excluded from the final analysis in women who required treatment in addition to that indicated in the original protocol. Cases of particular interest, are, however, described in detail

in appendix J.

The results are interesting in that a high proportion of pregnant women seem to show evidence of sub-clinical deficiency of iron and/or folic acid. There also seems to be a close relationship between iron and folic acid metabolism.

This work is considered to be of value for two reasons:-

- 1) The time seems to be rapidly approaching when a project of this sort might no longer be considered ethical and it is necessary to lose no opportunity of increasing our knowledge of normal physiology.
- 2) The results demonstrate some features not previously noted. These require further investigation to extend our understanding of the relationship of iron and folic acid to each other and to erythropoiesis and plasma volume changes.

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This work was done while holding a Research Fellowship in the Department of Obstetrics of the Welsh National School of Medicine. The appointment was made by the Research Committee of the United Cardiff Hospitals. The work would not have been possible without the support of these bodies.

It is a pleasure to acknowledge assistance and advice given by many people.

The original protocol was drawn up in collaboration with Dr. Lewis Fanning who was at that time in charge of the Department of

Statistics of the Welsh National School of Medicine. Dr. Fanning was also responsible for the method of randomised selection used. In addition, his department also divided the iron and iron + folic acid tablets into numbered but otherwise unidentified bottles; and kept the the key until the end of the investigations.

The tablets were supplied free of charge by Lederle Laboratories.

The methods of serum iron and blood volume estimation were developed in collaboration with Dr. D. K. Watkins who was at that time Research Biochemist in the Department of Obstetrics, Welsh National School of Medicine. These methods have been published and reprints are supplied.

Imferon was used in estimating blood volumes and advice on dilution was given by Dr. L. E. Martin of Bengers Laboratories Limited.

Serum B₁₂ and serum folate estimations were made for me by Dr. J. L. Withey of the Department of Pathology, Welsh National School of Medicine.

Technical assistance was given at various times by Mr. J. Hopkins, Miss Brenda Morgan, Mrs. Molly Jones, Miss Susan Muxworthy and Miss Anne Fraser.

Advice and assistance with the statistical analysis was given by Dr. Hubert Campbell, Senior Lecturer in Statistics in the Welsh National School of Medicine and Dr. E. A. Murphy lately Assistant Professor in the Departments of Medicine and Epidemiology of the Johns Hopkins University Medical School.

The typing of the manuscript has been done by Miss Anne Townsend and Mrs. Lesley Viggers.

The scattergrams and graphs were perfected and photographed by the Department of Medical Illustration of the Cardiff Royal Infirmary and the photographs were mounted by Miss Anne Fraser.

The inception and progress of the work owes much to the encouragement of Professor A.S. Duncan at that time Professor in Obstetrics and Gynaecology at the Welsh National School of Medicine. His successor, Professor A.C. Turnbull has also supported the final stages of composition.

It has been of great value in my understanding of the problems involved to discuss the work as it progressed with Dr. A. Jacobs, Senior Lecturer in Haematology in the Welsh National School of Medicine and also with Dr. A.D.T. Govan, Director of the Research Department, The Royal Maternity Hospital, Glasgow, and Dr. Jean Scott of the same Department.

Finally, I must express my thanks to the 154 women without whose co-operation in submitting themselves to many investigations this work could never have been done.

Chapter I.

Introduction

'It is quite certain that reproduction is not in itself a pathological process, and if pregnancy and lactation seem to require inevitably routine mass therapeutic measures, then we may be sure that something is wrong with modern living, or in the interpretation of our information.' Garry and Wood, 1946.

This observation of Garry and Wood is often quoted,

but it has to be accepted that anaemia, usually due to iron deficiency, is very common in women in this country. It is most prevalent between the ages of 15 and 44 years (Kilpatrick, 1961). This is the reproductive period and also a time when iron balance is liable to be precarious because of menstrual loss. It has been shown that over a third of normal women have a blood loss of 40 ml per month or more which is equivalent to an average daily iron loss of more than 0.6 mg per day (Jacobs & Butler, 1965). A relatively high prevalence of iron deficiency anaemia persists after the menopause and this has been described as a chronic anaemia starting during the child-bearing years (Kilpatrick & Hardisty, 1961).

Routine administration of iron during pregnancy has only come into common use in the last 10 or 15 years and is still not universally accepted as being of value in normal pregnancy. Hytten & Leitch (1964) could find no convincing published evidence that it was an advantage to a normal pregnant woman to take extra iron. They agreed with Fisher & Biggs (1955) that the real benefit is probably that '....the confidence of both doctor and midwife, particularly in domiciliary practice, is greatly increased by

by the knowledge that the haemoglobin level is high at term.'

Anaemia, or at least illness which responded to treatment with iron, is not a modern disease. Iron was one of the few inorganic medicaments described in the early Egyptian pharmacopoeias (Hoppe et al 1955). Celsus gave patients with splenomegalic anaemia water in which red hot irons had been drenched (D'Arcy & Howard, 1962). Therapeutic use of iron in the early centuries was empirical and probably related to the association of iron with the god Mars. With the development of Christianity the attributes of the gods tended to be transferred to the planets of the same name. By the eighteenth century a more scientific approach to medicine was developing and in 1746 Menghini showed that the iron content of blood could be increased by the ingestion of food rich in iron (Haden, 1938).

It would seem that severe iron deficiency anaemia was very common, particularly in girls at the time of puberty. This condition was first described by Johannes Lange in 1554 in an article entitled 'De Morbo Virgineo', (Lancet, 1941) but it was not until 1832 that Fodisch showed that the blood of these patients was deficient in iron (D'Arcy and Howard 1962). Chlorosis was discussed in Allbut and Rollestons System of Medicine (1912) and was described as being almost inevitable at puberty and likely to occur in all women at some period of their lives. By 1924 McGowan reported a recent decrease in the condition and attributed this to better understanding of diet and the nearer approximation of the life of women to that of men.

Incidental comments made in papers published in the nineteenth and early twentieth centuries suggest that in pregnancy blood levels were

accepted as normal which would now be looked upon as reflecting moderate or even severe anaemia. Willcocks (1881) quotes the haemoglobin levels given by Cazeaux in his *Traite de l'Art des Accouchements*. In a normal woman the level was given as 120 g per 1,000 ml falling to 67.7 per 1,000 ml in pregnancy. It is of course, possible that these values are not equivalent to modern haemoglobin estimations. Osler (1919) also refers to the 'usual' picture of fall in red cells, low haemoglobin and slight leucocytosis in pregnancy. It is therefore, not surprising that papers dealing with anaemia in pregnancy describe very severe and often fatal cases. It is often difficult to interpret the anaemias described in modern terms. Many are related to haemorrhage possibly accentuated by long-standing iron deficiency and others with acute sepsis which has no modern equivalent. It is also possible to recognise very severe megaloblastic anaemia of pregnancy. These cases were usually recognised in the puerperium although symptoms in late pregnancy were often described (Hoskin & Ceiriog-Cadle, 1927). Osler (1919) recognised the condition as an Addisonian type of anaemia but unlike primary pernicious anaemia in that patients could recover without recurrence. He quotes Channing (1842) as being the first to describe the condition. This work preceded the classical descriptions of Addison and Biermer.

Chlorotic conditions of the blood were successfully treated with iron during the nineteenth century. In 1832 Blaud introduced treatment with pills containing ferrous sulphate and potassium carbonate. At the end of the century iron fell into disrepute as a therapeutic agent. Bunge in 1895 reported that inorganic iron was converted to iron sulphide which was not

absorbed from the intestine and Quinke and Van Noorden began to recommend very small doses on the assumption that large amounts of iron could not be utilised. This proved ineffective and mineral iron by mouth was thought to have little value (Haden, 1938). It seems probable that the decrease in severe chlorosis at this time reduced interest in iron therapy but it was still recommended by the older physicians such as Osler (1919) and Larrabee (1925).

The period between the two world wars was a time when the importance of dietary iron was emphasised. The work of Whipple, Robscheit and Hooper in 1920, in which they studied artificially induced anaemia in animals, was misleading. This was also the time of the introduction of the Minot - Murphy diet for pernicious anaemia (1926). It was not until 1936 that Witts introduced ferrous sulphate as specific treatment in hypochromic anaemia.

The first quarter of the present century was, however, important in that the concept of ante-natal care was established. The first pre-natal clinic was established by Dr. J. W. Ballantyne at the Edinburgh Royal Infirmary in 1901. Systematic ante-natal care did not begin until 1911 when clinics were set up in Leeds and Birmingham. It is interesting to note that in the controversial literature of the time the advantages put forward for the system cite detection of syphilis, toxæmia and mechanical abnormalities as being of primary importance. Prevention of anaemia has no place except under the general heading of "attention to diet and general hygiene." Treatment of anaemia in pregnancy would seem to have followed the same pattern as the treatment of anaemia in the general population as it was also in 1936 that Corrigan & Strauss reported the successful

treatment of pregnancy anaemia with ferrous sulphate.

Iron was now accepted as specific treatment for iron-deficiency anaemia in pregnancy and, with the work of Moore et al (1945), folic acid was established as being specific in megaloblastic anaemia of pregnancy.

The concept of prevention of anaemia in pregnancy by the use of routine supplements was a later development although in the case of iron this was advised by Hamilton and Wright in 1942. Among others Holly in America, Lowenstein and his colleagues in Canada and Giles and his associates in this country have been prominent in advocating the importance of routine treatment, begun early in pregnancy, to prevent the development of anaemia. In 1964 the British Medical Journal devoted one of the series 'Today's Drugs' to a consideration of 'Prophylactic Iron and Folic Acid in Pregnancy'. They concluded that iron should be given to all pregnant women throughout pregnancy and suggest that if this was enforced "much ill-health in middle life would be prevented and vast numbers of women saved from leading 'sub-optimal' lives". They also comment on the association of increased incidence of megaloblastic anaemia with severe iron deficiency (Chanarin et al, 1965) and recommend that in areas where megaloblastic anaemia is common that routine folic acid should be given in addition to iron.

When mineral and vitamin supplements are given it is necessary to consider the possibility of toxic effects due to excess. Severe reactions with iron are usually associated with the presence of free iron in the plasma which is not combined with transferrin. This rarely occurs

when iron is given by mouth because of the mucosal block (Iron Toxicity, 1955). Fatal results are only reported in young children who gain access to iron prescribed for adults (Committee on Toxicology report, 1959). Minor degrees of toxicity as demonstrated by nausea, vomiting, abdominal pain and diarrhoea are not uncommon but these symptoms do not seem to occur equally with different iron preparations in any individual.

There are also possible risks when folic acid is given.

Very rarely sensitivity reactions are reported (Mitchell et al, 1949; Chanarin et al, 1957; Woodliff & Davies, 1966) and Grønbaek and Larsen (1963) have described the development of polycythaemia vera after two months on a large dose of folic acid. The condition resolved itself when smaller doses were given. There is also the more important risk of folic acid being given as the only medication in a patient who in fact has primary vitamin B₁₂ deficiency anaemia. The risk is not great in pregnancy as a women with any significant degree of Vitamin B₁₂ deficiency is unlikely to be pregnant. (Today's drugs, 1964). An interesting case is however, reported by Varadi (1964). A multiparous woman was diagnosed as having pernicious anaemia during pregnancy and aborted at 26 weeks. Following treatment with vitamin B₁₂ she had a successful 11th pregnancy.

The risks of ill-effects when routine supplements are given are therefore not particularly great when weighed against the positive advantages which are the result of prevention of anaemia. In addition to the promotion of good health in the mother it has been shown that there is a strong correlation between stillbirth rates and maternal anaemia. There is also an increased incidence of prematurity (Klein, 1962; Macgregor 1963).

Folic acid deficiency even when it is not severe enough to cause megaloblastosis has been shown to be a disadvantage in pregnancy. Martin et al (1965) have described low folate levels associated with recurrent abortion. These authors advise treatment with folic acid before the woman embarks on another pregnancy. Congenital malformations have been reported in folate deficient rats (Nelson, 1960). Hibbard & Smithells (1965) investigated the incidence of folate deficiency as shown by a positive Figlu test in women who delivered normal infants. There was an incidence of positive Figlu tests in 65% of women who delivered malformed infants in contrast to a 17% incidence in the paired controls. Hourihane et al (1960) and Hibbard & Hibbard (1963) have also described an increased incidence of abruptio placentae in women with folic acid deficiency.

Many people are, therefore, in favour of giving supplements of iron with or without additional folic acid to all pregnant women. It therefore becomes important to study the effect of these supplements on apparently normal women. It is also desirable to discover what proportion of women do not need protection against sub-clinical or frank deficiency and whether it is possible to identify such women early in pregnancy. More information is also needed on the effect of iron in excess of normal requirements on the physiological adaptation to pregnancy. There is also insufficient information on the way in which additional folic acid will modify the effect of iron.

In the presentation which follows volume changes and the status of iron and folic acid nutrition are studied in the absence of treatment and in relation to treatment with iron or with iron and folic acid.

Chapter 2

Materials and Methods

Patients Studied

The women who took part in the investigations were patients with a haemoglobin level above 10.0g% who attended the booking clinics at the Maternity Hospital, Glossop Terrace, Cardiff before the 20th week of pregnancy. These women were interviewed to explain the object of the investigation and the procedures which would be involved. They were then asked if they would co-operate.

Patients were recruited for the project between December 1962 and October 1963, during which time there was a considerable amount of publicity with reference to foetal malformations following the administration of thalidomide in early pregnancy. It seems probable that this explains the unexpectedly high refusal rate which was 39% of the women seen. A further 18% were excluded for medical reasons.

During the period March 14th-October 18th, 1963, a detailed record was kept of the medical reasons for exclusion and also the reasons given by patients to justify their refusal. Three hundred and twenty-eight women were seen during this period and 61 were excluded for various causes among which urinary infection and threatened miscarriage were the commonest. A further 127 women refused to take part; in 36 cases either the husband or wife objected and in 49 cases this was expressed as "family difficulties." (Appendix A, Volume 2, Page 2). At the commencement of the work it was found that objections to the investigation most commonly came

from the husband. An explanatory letter was therefore prepared for the wife to take to her husband and there was little further trouble.

A copy of this letter is included in the pocket at the back of Volume 2.

There was no significant difference in age or parity between those who entered the investigation and those who did not (vol. 2 p. 15) but one gained a strong clinical impression that those who agreed to co-operate were more intelligent and that many were from the higher social classes.

Patients who entered the investigation were placed in one of three groups. The method of selection was worked out by Dr. Lewis Fanning on a statistical basis to ensure an even spread of women with regard to age, parity and initial haemoglobin level. The first group received iron in the form of ferrous sulphate 194 mgs. (61 mgm. elemental iron) twice daily after meals. In the case of the second group the ferrous sulphate tablets also contained 1.7 mgm/folic acid; these were also given twice daily. The third group were initially untreated. The tablets in the treated groups were identical in appearance and were made up in numbered bottles to correspond with the numbers allotted to the patients in the trial. The code was not disclosed until the end of the investigation. It was decided not to give the untreated group a placebo as it seemed preferable to know which women were untreated so that there would be no delay in starting therapy if this became necessary. In all cases treatment was discontinued after delivery.

Two hundred women entered in the investigation, 68 on iron, 68 on iron and folic acid and 64 untreated, but only 154 were followed through to the post-natal visit. The groups remained evenly divided (iron 49, iron and folic acid 51, untreated 54) and there was no significant difference between the groups for age, parity or initial haemoglobin level (vol. 2 p. 15).

Eight women were excluded for medical reasons such as confusion over therapy, intercurrent illness and difficult veins. Six were possibly intolerant to imferon but their discomfort could well have been nervous in origin. One woman had a definite reaction to imferon at the second blood volume estimation (see Appendix J). Among the remaining 31 who failed to complete the investigation, 5 miscarried or went into premature labour, 11 were intolerant of the form of iron used and fifteen failed to attend. (vol. 2, p. 3).

Patients entered the investigation at 16 weeks or 20 weeks and in all patients, haemoglobin and haematocrit estimations were done and a blood film examined at monthly intervals throughout pregnancy and also at the post-natal visit 6-8 weeks after delivery. The blood film was assessed for evidence of anaemia and a mean lobe count made on 100 polymorphonuclear leucocytes. In addition the percentage of polymorphonuclear leucocytes with 5 or more lobes was noted. Serum iron and total iron binding capacity were also estimated on all patients at 20 weeks 28, 36 and 40 weeks and at the post-natal visit.

Serum B₁₂ and serum folate estimations were made on half of the women in each of the 3 main groups at monthly intervals from 16 or

20 weeks (dependant on the time of entering the investigation). While in the other half, blood volume estimations were made at 20, 28, 36 and 40 weeks. The investigations were repeated in both sub-groups at the post-natal visit. When the blood volume was estimated, extra serum was collected at 20 weeks, 36 weeks and post-natally. This was stored at -20°C until the three specimens were obtained and then protein electrophoresis strips were run simultaneously.

It will therefore be seen that the women studied form six sub-groups identified as groups 1 to 6 depending on the treatment given and the nature of the main type of investigation.

<u>Group</u>	<u>Treatment</u>	<u>Investigation</u>
1	Iron	B. V.
2	Iron	Serum B ₁₂ & Folate.
3	Iron + Folic acid	B. V.
4	Iron + Folic acid	Serum B ₁₂ & Folate.
5	Initially untreated.	B. V.
6	Initially untreated.	Serum B ₁₂ & Folate.

Tables showing the detailed results of each investigation on all of the women are given in the appendices in Volume 2. The groups are identified by the numbers as shown above.

When the results were studied at the end of the investigation it was found that only 16 (30%) of the untreated group remained completely untreated throughout the pregnancy. This meant that there were in fact four groups to consider instead of the three groups of the original

protocol. These are shown below with the number of patients in each group and the lettered code by which they were identified.

I	Iron	49	Blood volume	20
			Serum B ₁₂ & folate	29
I+F	Iron + Folic Acid	51	Blood volume	24
			Serum B ₁₂ & folate	27
Cu	Completely Untreated	16	Blood volume	8
			Serum B ₁₂ & folate	8
Cw	Patients requiring treatment while untreated.	38	Blood volume	17
			Serum B ₁₂ & folate	21

Patients in the Cw group were excluded after beginning treatment and none remained after 38 weeks.

In comparison with other workers in this country 30% represents a low rate of salvage for a control group. Chisholm (1966) in a similar investigation needed to treat 35 of 177 women (20%) who were initially given a placebo instead of iron. Her investigation, however, did not start until 28 weeks and 50 women were excluded from the study as already showing evidence of anaemia. Chanarin et al (1965) found that 10 women in a control group of 52 (19%) required iron therapy. My own figure is closer to that of Holly (1955) who considered that as many as 78% of women will develop at least mild iron deficiency anaemia during pregnancy if untreated.

The differences, to some extent, are explained by the criteria used

to decide when treatment becomes necessary. It was originally intended not to give iron therapy in the untreated groups until the haemoglobin level fell below 10.0 g%. In practice it was found that treatment was started at 11.0 g% and because of the method of haemoglobin estimation used (see below) the final reading, taken after the patient had left, was often above this figure.

It would in fact have been difficult to treat these women as they took a great interest in the investigation and in their haemoglobin levels and were anxious that nothing should be done, or left undone, to prejudice the foetus. Complaints of fatigue and general lack of wellbeing were common in this group and improved when they were given oral iron. This might reflect lack of iron stores as described by Holly (1965) and Fielding et al (1965). Beutler and his co-workers (1960) showed that symptoms were relieved when iron was given to women with normal haemoglobin concentrations but with no evidence of stored iron as shown by examination of the marrow. Jennison and Ellis (1954) also reported resolution of symptoms in cases of iron deficiency anaemia within a few days of starting treatment and before the haemoglobin concentration began to rise. The more recent work of Elwood and Wood (1966) does not confirm this. Iron or a placebo was given to women with a haemoglobin of more than 10.0 g% and although there was a significant rise in haemoglobin level in patients on iron, there was no convincing evidence of an improvement in any symptom. A preliminary analysis of a further study on women with symptoms of "chronic fatigue" and haemoglobin levels of more than 13.5 g% suggests that iron gave no

greater benefit than a placebo (Elwood 1967, personal communication).

It is therefore accepted that a proportion of the women in the Cw group were at most only slightly anaemic and it seems likely that if placebo tablets had been given more women would have remained untreated.

The Cu group, however, now consists of women, who from the haematological point of view, probably approximate much more closely to the ideal of normality than is usual in most control groups in the literature.

Methods

The methods used in the various investigations are outlined and discussed here but full technical details are given in Volume 2, Appendix I.

Haemoglobin was estimated in triplicate, using capillary blood, by the alkaline - haematin method as modified by Dr. J.L. Withey (unpublished). A preliminary reading was available at the time when the patient was seen at each visit, but for purposes of the study the level given after the alkaline - haematin mixture had stood for 15 minutes was used. The mean value for the three specimens was taken.

Capillary blood was also used to estimate the haematocrit by means of the Hawksley micro-centrifuge. Estimations were again made in triplicate and the mean taken.

The mean corpuscular haemoglobin concentration was calculated from the haemoglobin and haematocrit readings.

Blood films were made directly from capillary blood and stained with Leishman's stain. The films were assessed as being

normochromic or hypochromic, normocytic, microcytic or macrocytic and for the presence or absence of poikilocytosis and polychromasia. The number of lobes in 100 polymorphonuclear leucocytes was counted and the mean taken. The number of polymorphonuclear leucocytes with 2, 3, 4, 5 and 5+ lobes was also noted and a general impression of the appearance of the film given.

Bone marrow was only examined when necessary for diagnostic purposes and on these occasions sternal puncture was done.

Serum B₁₂ and serum folate estimations were done in the Welsh National School of Medicine, Department of Pathology by courtesy of Dr. J. L. Withey.

Serum B₁₂ was assayed with euglena gracilis (Z strain) using a method modified from Ross (1952) and Hutner et al (1956). Normal range 120-650 µg/ml.

Serum folate was assayed using the lacto-bacillus casei method of Waters and Mollin (1961). Normal range 2.1-20 µg/ml.

A micro-method for the determination of serum iron and total iron binding capacity was devised for the purpose of this investigation in collaboration with Dr. D. K. Watkins (Watkins and Butler 1966). The process made use of a scaled down and slightly modified version of the method described by Henry et al (1958) for unsaturated iron binding capacity.

The micro-method for serum iron estimation was also applied in the plasma volume studies as the estimations were made by injecting the iron dextran complex "Imferon". (Butler and Watkins 1966).

It was fully appreciated that the most accurate method for measuring blood volumes are those in which plasma and red cell volumes are estimated individually. Estimations of red cell volume, however, involve the use of radio-active tracers such as ^{51}Cr . (Gray and Sterling, 1950; Strumia et al 1958) or ^{32}P (Reave and Veal 1949; Verel et al 1956), and although this has been done in pregnancy (Paintin 1963 and Pritchard 1965) it seemed preferable not to risk the possible effect on the foetus.

The use of Evans Blue (T-1824) (Gibson and Evans 1937; Mollison 1961) is a well established method of plasma volume estimation, but as serum iron estimations were already being performed, it seemed to be an advantage not to increase the number of procedures to be perfected by a limited technical staff.

The use of Imferon to estimate plasma volume was first described by Scott (1956) and was later used by Mackenzie and Tindle (1959). The method used in this study differed from that described by the previous authors in that the amount of Imferon given was reduced to the equivalent of 20 mgm of elemental iron compared with 100 mgm given by Scott (1956) and 50 mgm given by Mackenzie and Tindle (1959).

Blood volumes and red cell volumes were calculated making use of the haematocrit reading. It is now universally accepted that the venous haematocrit differs from the true body haematocrit calculated from plasma volume and red cell volume by about 10%. This is because there is a higher proportion of plasma to red cells in smaller blood vessels than in larger blood vessels (Fahreus 1929). The ratio between body haematocrit and

venous haematocrit is called the haematocrit ratio. The mean haematocrit ratio was shown to be constant in non-pregnant individuals. It was calculated as 0.91 by Gibson and his co-workers (1946) and also by Chaplin et al (1953). Gibson et al estimated red cell volume using cells labelled with either ^{55}Fe or ^{59}Fe while Chaplin and his colleagues used red cells labelled with ^{32}P . Both groups estimated plasma volume using Evans Blue. Caton and his colleagues (1951) repeated this work in pregnancy, estimating plasma volume with Evans Blue and red cell volume with cells labelled with ^{55}Fe , but they found that the haematocrit ratio varied and came close to unity as term was approached. Similar results were reported by Verel et al (1956). These findings have not been confirmed by recent work. Pritchard and Rowland (1964) used ^{131}I albumen to estimate plasma volume and ^{51}Cr tagged red cells to estimate red cell volume. They found the mean haematocrit ratio to be 0.89 in non-pregnant women, women in late pregnancy and also 48 hours after delivery. Similarly Paintin (1963) using Evans Blue and ^{51}Cr tagged red cells found that the haematocrit ratio was sufficiently constant during pregnancy to be used to calculate total blood volume and red cell volume from observed plasma volume. The mean haematocrit ratio in Paintin's series was given as 0.88 and this figure was used for the purpose of the present project.

In earlier work on blood volume estimations it was also considered necessary to correct the haematocrit for trapped plasma, but in this study haematocrit readings were done using the Hawksley micro-centrifuge (12,000 g for 5 minutes) and this correction was not considered to be necessary (Paintin 1963).

It was however, found that there was liable to be a fall in the haematocrit readings, of up to 2% in some women, between the specimens taken before and after giving Imferon. Preliminary work indicated that the variations were individual and were associated with posture (Mollison 1961). It was not possible to arrange for the women to rest for 30 minutes in a warm bed before making the blood volume estimations although this would probably have eliminated the difficulty, (Paintin 1962). The estimations had to be made when patients attended the ante-natal clinic. Haematocrit readings were therefore taken before giving Imferon and also on the post-Imferon specimen. When there was a change in the haematocrit, the plasma volume was corrected to that corresponding to the first result, assuming that the red cell volume was constant throughout (Butler and Watkins 1966.)

Blood volume estimations were made throughout pregnancy and at the post-natal visit on 52 women. In 38 of these cases additional blood was taken at 20 weeks, at 36 weeks and at the post-natal visit for protein electrophoresis. The serum was separated and stored at -20°C until the group of specimens for each patient was complete. They were then run simultaneously. The method used was that of Sammons and Whitehead (1963).

The intention was to compare the changing pattern of the protein fractions between the middle and end of pregnancy. It was therefore thought to be sufficient to express the albumen and globulin constituents as a percentage of the whole and total protein was not estimated.

All specimens were taken when patients attended the ante-natal clinics so that there was no uniformity in the time of day at which specimens

from different women were taken but there is reasonable uniformity between the successive specimens from individuals.

Chapter 3

Observations: 1. Haemoglobin Concentration and Iron Metabolism

The results of the investigation will be described and discussed in the next three chapters. Comparisons will be made between four groups of women:-

Patients on Iron	I
Patients on Iron + Folic Acid	I+F
Completely Untreated	Cu
Requiring treatment while untreated	Cw

The variables are considered at 20, 28, 36 and 40 weeks and also at the post-natal visit 6-8 weeks after delivery. The mean values for each group are compared and also in most instances the mean regression co-efficient during pregnancy (post-natal readings were excluded). Where relevant Fisher's method for combining significance levels from different samples is also applied.

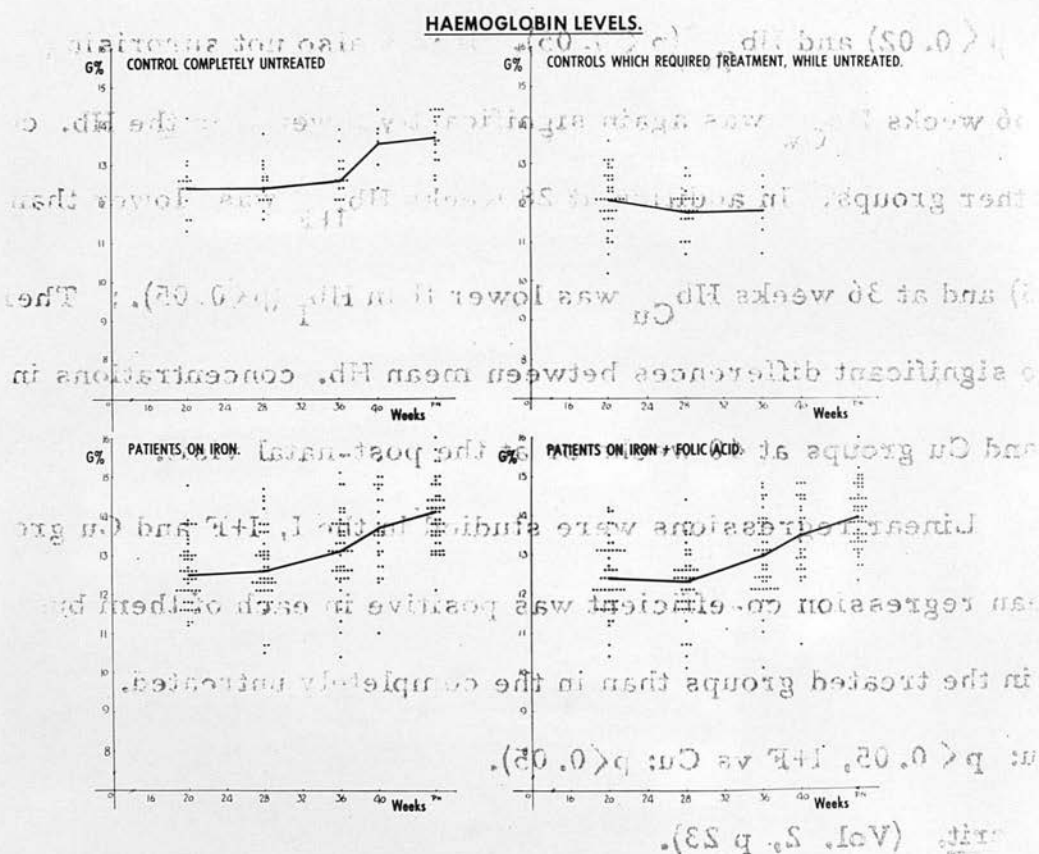
RESULTS

Haemoglobin Concentration (Vol. 2, p. 16 and Figure 1).

Mean Values and Range (g%)
(Number of estimations in brackets)

Weeks	I	I +F	Cu	Cw
20	12.6 (49) 11.2-14.8	12.4 (51) 10.4-14.2	12.5 (16) 11.3-14.2	12.1 (38) 10.2-13.6
28	12.6 (49) 10.5-14.7	12.4 (51) 10.1-14.4	12.5 (16) 11.6-13.8	11.8 (25) 10.1-12.9

Fig. 1. Haemoglobin; Scattergrams and Mean Values



Weeks	I	I + F	Cu	Cw
36	13.1 (48) 10.4-14.8	12.9 (49) 10.1-14.8	12.6 (16) 11.8-13.9	11.8 (9) 10.7-12.4 ⁺
40	13.6 (27) 11.0-15.4	13.3 (33) 10.7-14.8	13.5 (6) 12.4-14.4	-
PN	14.0 (49) 12.1-15.7	13.9 (51) 12.3-16.0	13.8 (16) 12.1-14.7	-

Hb. ^{*} Cw was significantly lower at 20 weeks than Hb_I ($p < 0.005$), Hb_{I+F} ($p < 0.02$) and Hb_{Cu} ($p < 0.05$). It was also not surprising that at 28 and 36 weeks Hb_{Cw} was again significantly lower than the Hb. concentration in the other groups. In addition at 28 weeks Hb_{I+F} was lower than Hb_I ($p < 0.05$) and at 36 weeks Hb_{Cu} was lower than Hb_I ($p < 0.05$). There were no significant differences between mean Hb. concentrations in the I, I+F and Cu groups at 40 weeks or at the post-natal visit.

Linear regressions were studied in the I, I+F and Cu groups. The mean regression co-efficient was positive in each of them but was higher in the treated groups than in the completely untreated. (I vs Cu: $p < 0.05$, I+F vs Cu: $p < 0.05$).

Haematocrit. (Vol. 2, p 23).

Mean values and Range (%)
(Number of estimations in brackets)

Weeks	I	I+F	Cu	Cw
20	36 (49) 31-43	36 (51) 31-40	36 (16) 33-40	35 (38) 30-40
28	36 (49) 31-43	35 (51) 29-40	36 (16) 33-38	35 (25) 33-39

+ Excluding one patient (Hb. = 13.1 g%) who started treatment following Delivery because of heavy blood loss.

* Mean value in the group indicated.

Weeks	I	I + F	Cu	Cw
36	38 (48) 30-44	37 (48) 32-44	37 (15) 33-41	35 (9) 33-40
40	39 (26) 32-42	38 (33) 32-42	39 (6) 35-43	-
PN	49 (49) 34-45	40 (51) 35-44	39 (16) 35-42	-

The mean haematocrit level in the Cw group at 20 weeks was significantly lower than in the I group ($p < 0.02$) or the Cu group ($p < 0.02$). This difference was again noted at 28 weeks ($p < 0.002$). In addition at 28 weeks the mean haematocrit in the I+F group was significantly lower than in the I group ($p < 0.02$). At 36 weeks Haematocrit_{Cw} was significantly lower than the Haematocrit_I ($p < 0.02$). The mean values at 40 weeks were of interest as Haematocrit_{I+F} was lower than Haematocrit_I at the 5% level of significance. No significant differences were found between the mean post natal readings.

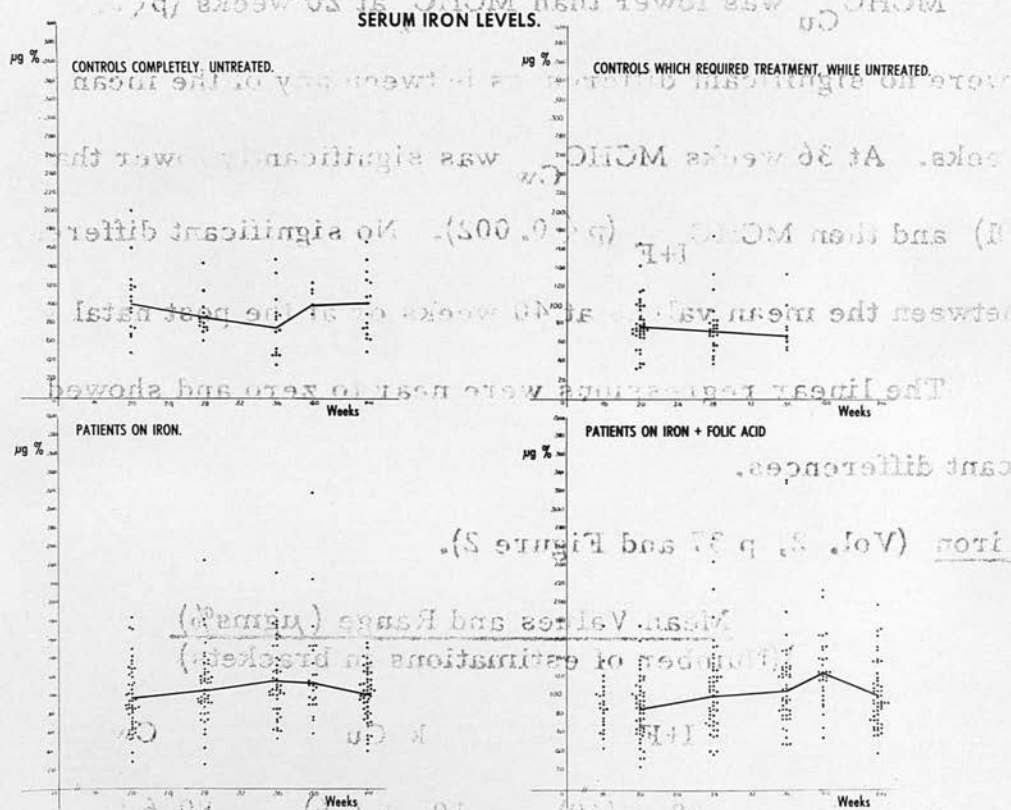
The mean regression co-efficient was positive in the three groups studied and was significantly higher in the group on Iron than in the completely untreated group ($p < 0.0005$). It was also higher in the group on Iron + Folic acid than in the completely untreated group ($p < 0.05$).

Mean Corpuscular Haemoglobin Concentration (Vol. 2, p. 30)

Mean Values and Range (%)
(Number of estimations in brackets)

Weeks	I	I + F	Cu	Cw
20	35.1 (49) 31.1-38.7	34.9 (51) 31.0-39.0	34.3 (16) 32.7-36.5	34.8 (38) 31.4-37.7
28	34.8 (49) 31.5-38.0	34.8 (51) 31.0-38.7	34.5 (16) 32.5-35.7	34.2 (25) 31.5-37.0

Fig. 2. Serum Iron; Scattergrams and Mean Values



Weeks	I	I + F	Cu	Cw
36	34.7 (48) 31.2-39.3	34.4 (48) 32.3-39.0	34.5 (15) 33.0-36.5	33.2 (9) 29.0-35.5
40	35.1 (26) 31.2-38.6	35.2 (33) 32.5-37.3	34.4 (6) 32.5-35.5	-
PN	35.0 (49) 31.5-38.3	35.1 (51) 31.2-38.4	34.9 (16) 33.2-36.3	-

$MCHC_{Cu}$ was lower than $MCHC_I$ at 20 weeks ($p < 0.05$).

There were no significant differences between any of the mean values at 28 weeks. At 36 weeks $MCHC_{Cw}$ was significantly lower than $MCHC_I$ ($p < 0.01$) and then $MCHC_{I+F}$ ($p < 0.002$). No significant differences were noted between the mean values at 40 weeks or at the post natal visit.

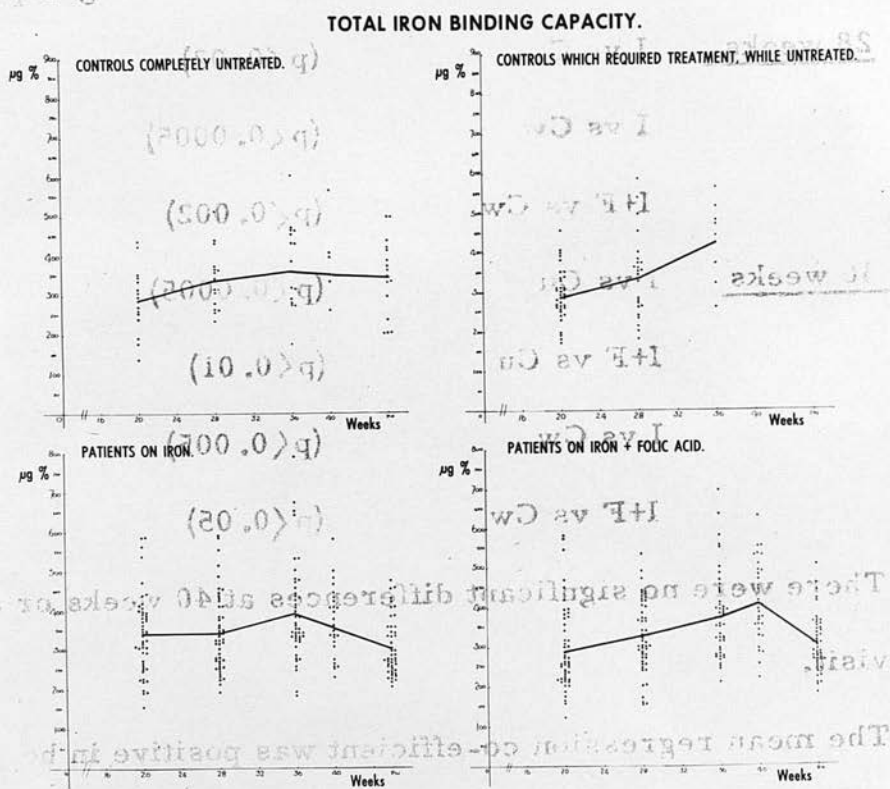
The linear regressions were near to zero and showed no significant differences.

Serum iron (Vol. 2, p 37 and Figure 2).

Mean Values and Range ($\mu\text{gms}\%$)
(Number of estimations in brackets)

Weeks	I	I+F	k Cu	Cw
20	97.5 (48) 39.4-184.0	88.1 (49) 25.0-176.0	106.9 (16) 50.5-202.0	80.6 (38) 35.7-168.5
28	110.2 (48) 27.0-246.0	109.2 (50) 39.4-270.0	88.2 (15) 63.0-146.0	78.2 (24) 39.4-176.0
36	118.3 (45) 54.0-232.0	115.6 (48) 49.0-331.0	78.5 (15) 37.0-150.0	78.2 (9) 54.0-135.0
40	124.4 (26) 60.5-319.0	127.8 (32) 56.5-215.0	109.8 (5) 91.0-125.0	-
PN	101.8 (47) 40.6-157.5	100.9 (50) 39.4-199.0	100.7 (16) 50.5-169.0	-

Fig. 3. Total Iron Binding Capacity; Scattergrams and Mean Values



At 20 weeks Serum Iron_{Cu} was significantly higher than Serum Iron_I ($p < 0.002$), Serum Iron_{I+F} ($p < 0.05$) and Serum Iron_{Cw} ($p < 0.005$). The mean value in the group which would require treatment was also significantly lower than the mean value of the group on Iron ($p < 0.01$). The mean serum iron levels at 28 and 36 weeks, were significantly higher in the groups receiving treatment (with the exception of I+F vs Cu at 28 weeks) than in either of the untreated groups:-

<u>28 weeks</u>	I vs Cu	($p < 0.02$)
	I vs Cw	($p < 0.0005$)
	I+F vs Cw	($p < 0.002$)
<u>36 weeks</u>	I vs Cu	($p < 0.0005$)
	I+F vs Cu	($p < 0.01$)
	I vs Cw	($p < 0.005$)
	I+F vs Cw	($p < 0.05$)

There were no significant differences at 40 weeks or at the post-natal visit.

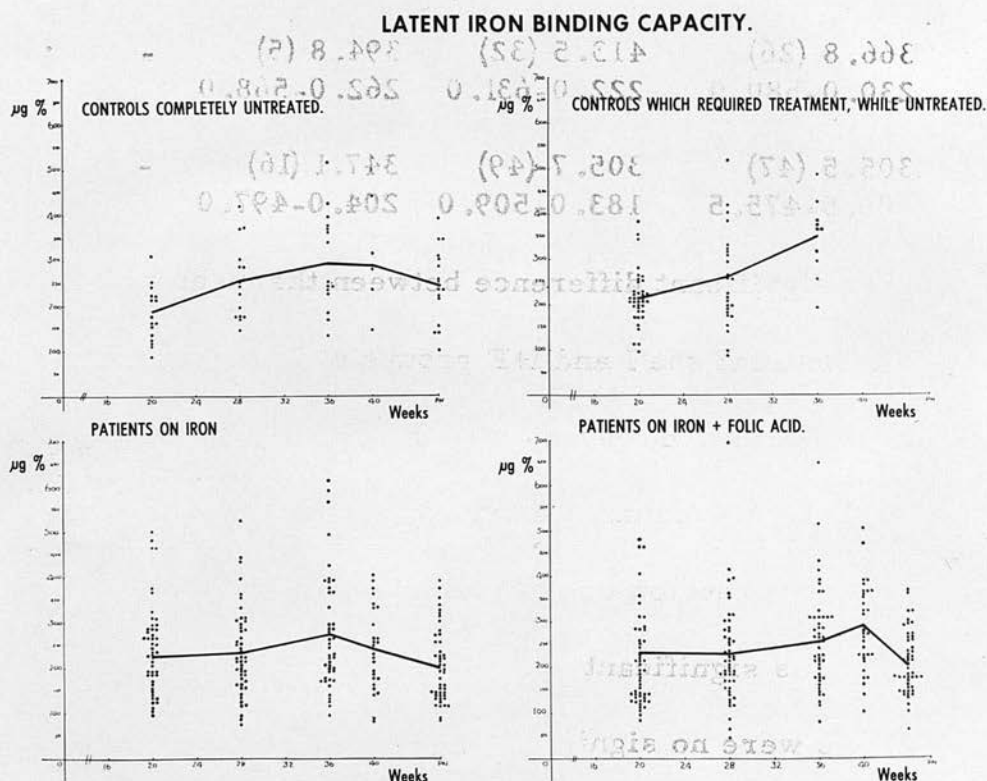
The mean regression co-efficient was positive in both of the treated groups and negative in the completely untreated group. These differences were highly significant (I vs Cu, $p < 0.002$ and I+F vs Cu, $p < 0.0005$). The positive linear regressions were also significant as judged by Fisher's test in both of the treated groups ($p < 0.01$).

Total Iron Binding Capacity (Vol. 2 p. 44 and Figure 3).

Mean Values and Range ($\mu\text{gms } \%$)
(Number of estimations in brackets)

Weeks

Fig. 4. Latent Iron Binding Capacity. Scattergrams and Mean Values



Weeks	I	I+F	Cu	Cw
20	343.8 (48) 156.5-586.0	294.7 (49) 123.4-548.5	290.4 (16) 139.5-438.0	294.4 (38) 172.0-458.0
28	345.2 (48) 192.0-590.0	336.8 (50) 156.5-782.5	341.7 (15) 236.5-512.0	331.9 (24) 143.0-588.5
36	393.1 (45) 182.0-790.0	378.1 (48) 211.5-699.0	377.4 (15) 179.8-604.5	429.3 (9) 261.0-565.0
40	366.8 (26) 230.0-580.0	413.5 (32) 222.0-631.0	394.8 (5) 262.0-568.0	-
PN	305.5 (47) 206.5-475.5	305.7 (49) 183.0-509.0	347.1 (16) 204.0-497.0	-

The only significant difference between the mean values at the weeks studied was between the I and I+F groups at 20 weeks ($p < 0.025$).

Total iron binding capacity, however, was the only variable which did not show uniformity on the Chi-square distribution at 20 weeks (vol. 2, p. 15).

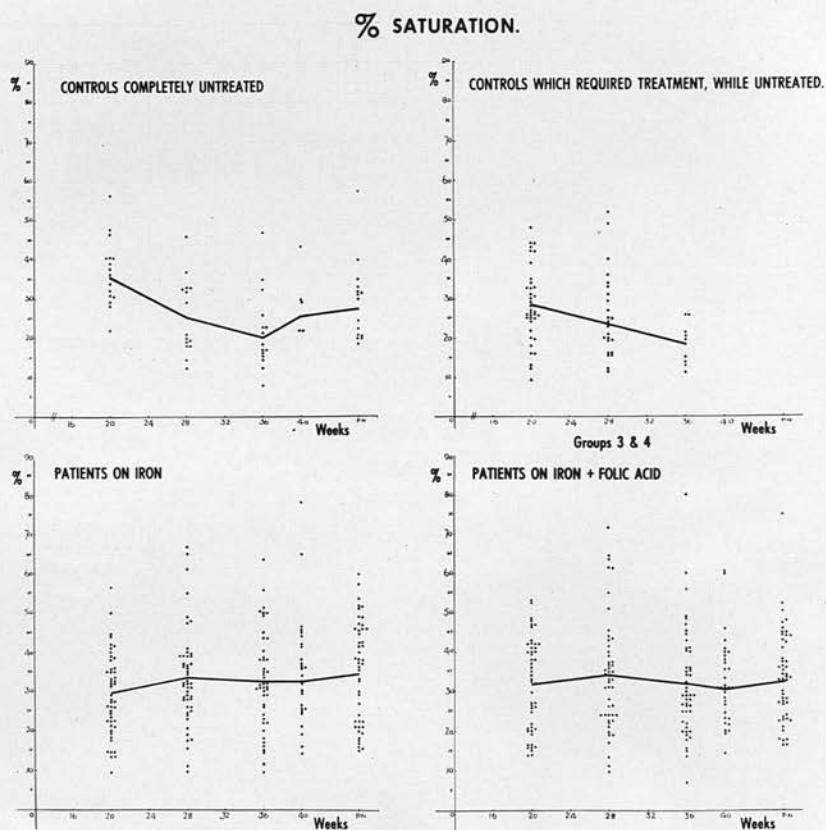
The mean regression co-efficient was positive in the three groups tested and the rise was significant by Fisher's test (I: $p < 0.025$, I+F: $p < 0.005$. Cu: $p < 0.05$). There were no significant differences between the groups.

Latent Iron Binding Capacity (Vol. 2, p. 51 and Figure 4).

Mean Values and Range (µgms %)
(Number of estimations in brackets)

Weeks	I	I + F	Cu	Cw.
20	246.1 (48) 96.0-500.0	206.6 (49) 81.4-465.0	183.5 (16) 89.0-310.0	215.1 (38) 96.0-384.0
28	235.4 (48) 74.0-525.0	227.6 (50) 44.5-695.0	253.4 (15) 170.0-420.0	253.7 (24) 87.5.-516.0
36	274.1 (45) 96.0-614.0	262.4 (48) 81.0-650.0	298.7 (15) 133.0-650.0	351.1 (9) 192.0-486.0

Fig. 5. % Saturation; Scattergrams and Mean Values



Weeks	I	I+F	Cu	Cw
40	242.4 (26) 81.0-405.0	285.7 (32) 103.0-509.0	285.0 (5) 148.0-443.0	-
PN	203.7 (47) 88.5-399.0	205.3 (49) 66.5-370.0	246.4 (16) 102.0-394.0	-

The mean latent iron binding capacity at 20 weeks was lower in the Cu group than in the other groups. This difference was significant when the comparison was made with the I+F group ($p < 0.02$). The only other significant difference between the mean values at the weeks studied was at 36 weeks when the group requiring treatment showed a higher mean value than the other groups. This was significant in the comparison of I+F vs Cw ($p < 0.025$).

Percentage Saturation (Vol. 2, p. 58 and Figure 5).

Mean Values and Range (%)

Weeks	I	I+F	Cu	Cw
20	29.5 (48) 13.5-50.0	31.0 (49) 13.9-53.0	37.0 (16) 22.0-56.5	28.3 (38) 9.7-48.0
28	33.4 (48) 9.7-67.0	34.1 (50) 9.9-71.5	27.2 (15) 14.5-46.0	25.7 (24) 11.5-52.0
36	32.7 (45) 9.6-63.6	31.8 (48) 7.0-80.0	21.8 (15) 8.0-47.0	18.6 (9) 11.2-26.0
40	35.2 (26) 14.3-78.3	32.4 (32) 14.5-60.6	29.4 (5) 22.0-43.5	-
PN	35.4 (47) 15.0-60.0	33.7 (49) 16.5-75.0	29.7 (16) 18.8-57.5	-

The mean value of the Cu group at 20 weeks was higher than the mean values in the other groups. The difference was significant

when the comparison was made with the group on Iron ($p < 0.01$) and with the Cw group ($p < 0.005$). At 28 weeks % Sat. C_w was significantly lower than % Sat. I or % Sat. $I+F$ ($p < 0.02$). As would be expected, at 36 weeks the mean % saturation in the treated groups was significantly higher than in the untreated groups

I vs C_u ($p < 0.005$).

$I+F$ vs C_u ($p < 0.01$)

I vs C_w ($p < 0.005$)

$I+F$ vs C_w ($p < 0.01$)

No significant differences were noted at 40 weeks or at the post-natal visit.

DISCUSSION

Haemoglobin Concentration

It is generally accepted that the haemoglobin concentration in pregnancy does not necessarily reflect the presence or absence of anaemia (Scott, 1962).

Willcocks (1881) recognised the occurrence of hydraemia in pregnancy due to the vascular system becoming filled with "fluid of liquor sanguinis" while the number of red cells remained constant. These views were confirmed when it became possible to estimate plasma volume (Dieckman & Wegner, 1934; Gibson & Evans, 1937).

Later observers showed an inverse relationship between haemoglobin levels and plasma volume during pregnancy. Lowest levels of haemoglobin concentration were found at the time of greatest plasma volume increase, that is between 28 and 36 weeks. Haemoglobin levels rose slightly

towards term (Evans, 1943; Scott and Govan, 1949; Giles and Burton, 1960) at which time most workers reported a fall in plasma volume (Lund 1951; Cope 1958). The term "physiological anaemia" was used to describe this condition of the blood in pregnancy and only levels below 10.0 g/100 ml were considered abnormal (Whitby & Britton 1953). In America Holly (1960) placed the level higher at 11.5 g/100 ml.

The concept of physiological anaemia has been questioned in recent years since it has been shown ~~that~~ when iron was given during pregnancy the fall in haemoglobin concentration could be corrected (Scott & Govan 1949; Kerr & Davidson, 1958). In addition treated cases showed a higher haemoglobin level after delivery (Verloop et al 1959). Recent writers have advised routine iron in pregnancy and have considered that a haemoglobin concentration of at least 11.8 g/100 ml should be maintained (Giles & Burton 1960).

Pregnancy is, however, a physiological state and one would expect a normal woman, on a normal diet, to adjust to it without mineral supplements. Hamilton (1950) has shown that haemodilution, by causing a relative decrease in cell elements, reduces blood viscosity and that this is associated with increased cardiac output. Relative increase of plasma volume also permits greater loss at delivery without the untoward effects which are seen when an equivalent rapid blood loss occurs in the non-pregnant.

The Cu group in the present study is of great interest in that they showed no evidence of anaemia. In this group the lowest mean haemoglobin level had been reached by 20 weeks. Analysis of results was begun at 20 weeks

as it was not possible to recruit sufficient women by 16 weeks. Results for a proportion of the patients, however, were available for this earlier stage of pregnancy. In the Cu group haemoglobin concentrations at 16 weeks were estimated in 11 of the 16 women (69%) and in the Cw group in 15 of the 38 patients (40%). In the first group the mean concentration was 13.2 g/100 ml with only 4 readings below 13.0 g/100 ml (36%) while in the second group the mean was 12.4 g/100 ml with 11 of the 15 readings below 13.0 g/100 ml (73%). These figures would suggest that in normal women the main fall in haemoglobin concentration due to haemodilution occurs between the 16th and 20th weeks of pregnancy. The mean haemoglobin concentration at the post-natal visit was 13.9 g/100 ml. in this group so that there had been a slight fall in the mean value by 16 weeks. This may reflect individual variation among the women studied. The high proportion of women attending by 16 weeks in the Cu group is also noteworthy and may be an indication of greater prudence in matters concerning health.

Perhaps the most important finding in this study of haemoglobin levels is that normal non-anaemic women show the same pattern and the same range of haemoglobin levels as women who are treated with iron alone or with iron + folic acid. There is no difference between the mean values in late pregnancy (36 weeks and later) or at the post-natal visit. Indeed slightly lower values are recorded in the group on iron + folic acid.

Eighty per cent of women however show an increased haemoglobin concentration when given iron in pregnancy (Kerr, & Davidson, 1958) and one has to consider whether this is sufficient evidence that these women were

anaemic. Iron has a pharmacological as well as a nutritional effect (Witts, 1962). This has been demonstrated by Paintin et al (1966). These workers compared the effect of low and high doses of iron in pregnancy. The low iron group received 12 mgms of iron daily in tablet form and it was anticipated that this would have shown some effect on haemoglobin concentration and red cell volume had the women been iron deficient. In practise this dosage had almost no effect. The high iron supplement (100 mgms per day) produced the type of response usually reported. When iron was given in therapeutic doses Paintin and his co workers noted a fairly uniform increase of red cell volume (160 ml). This suggested to them that iron, in large quantities, had a specific stimulating effect on erythropoiesis. The iron binding capacity remained high in this group indicating that in pregnancy an elevated total iron binding capacity is not an indication of iron deficiency. Stimulation of erythropoiesis with large doses of iron seems to occur only in pregnancy. Most workers report no effect when iron is given to normal men and normal non-pregnant women (De Leeuw et al, 1964). Beutler et al, however, found a response in women with normal haemoglobin levels but with bone marrow depleted of iron stores. Iron stores are commonly reduced in pregnancy (Holly & Grund 1959) and this, together with the major physiological changes which occur to ensure transfer of iron to the foetus and enlargement of the haemoglobin mass, may make possible the erythropoietic effect of a large iron supplement.

In the majority of the published series, in which iron in

therapeutic doses have been given to pregnant women, a proportion show no increase in haemoglobin levels and some may show a fall. In the series of Paintin et al (1966) 4 of the 56 women in the high iron group showed no increase in haemoglobin concentration. Scott and Govan (1949) divided their cases into three groups 40% who improved, 52% who showed no change and 8% who deteriorated. This finding has been variously explained:-

- a) Failure to take the tablets given
- b) Failure to absorb iron
- c) An unusually large increase in plasma volume (Whiteside 1960).
- d) A mild folic acid deficiency

The occurrence in some cases of mild and unsuspected folic acid deficiency in pregnancy has been accepted since the work of Chanarin et al (1959). Chisholm (1966) reported that in spite of the iron therapy 12% of Oxford women near term had haemoglobin concentrations of less than 11 g/100 ml. Overt megaloblastic anaemia due to folic acid deficiency was uncommon. The possibility that these women were reflecting a mild folic acid deficiency was investigated by comparing haemoglobin levels in late pregnancy between women on iron and women on iron + folic acid. There was no significant difference between these groups. Giles and Burton (1960) report a similar investigation in which a much larger group was of women studied and in which larger doses of folic acid were given (15 mgms/day as compared with 5 mgms/day). In their series the mean

- haemoglobin level at term in the group on Iron + Folic acid was 12.17 g/100 which was significantly higher than the mean level of 11.81 g/100 ml found in the control group who only took iron.

In the present investigation a rather smaller dose of folic acid (3.4 mgm/day) was given from an earlier stage in pregnancy. There was no significant improvement in haemoglobin levels in comparison with patients on iron only. Indeed at 28 weeks the mean haemoglobin concentration in the I+F group was 0.2 g/100 ml lower than in the group on iron and this was significant at the 5% level. The range was very similar in both groups.

It seems probable that the socio-economic conditions under which these women lived were more comparable with those of women in the Oxford area (Chisholm, 1966) than in Stoke on Trent (Giles & Burton, 1960). Inadequate diet is an important aetiological factor in the development of megaloblastic anaemia of pregnancy and folic acid deficiency (Lowenstein et al, 1966; Giles & Burton, 1960). It is therefore not surprising that Giles and Burton should report improvement in haemoglobin levels when additional folic acid was given to a population in which inadequate diets are common; or the failure to demonstrate this phenomenon in more fortunate areas.

Haematocrit Levels

In non-anaemic pregnant women the haematocrit levels parallel the levels of haemoglobin concentration. The completely untreated group in this investigation show similar mean values to those found in treated groups. The lowest mean value is 36% which is only slightly lower than the lowest mean of 36.7% reported by Rath et al (1950). Hytten and Duncan (1956) quote 34% as the average lowest haematocrit reading in normal pregnancy. Hytten and

Leitch (1964) comment on the similarity of the findings of Rath et al (1950) to those reported when iron had been given (Lund 1951). They suggest that the women may have been taking iron supplements of their own accord. Women in the Cu group in this series were carefully questioned on this point and were not taking iron. It would seem therefore, that a proportion of women are able to maintain a haematological balance in pregnancy with haemoglobin and haematocrit levels of the same order as those found in treated women. This will be discussed further under iron metabolism.

The mean haematocrit levels in the Cw group were slightly lower than the mean values in the other groups. It is however, of interest to note that at 28 and 36 weeks the values at the lower end of the range are higher than in the other groups. This was not observed with haemoglobin levels and is no doubt due to the increasing hypochromia in these patients.

Mean Corpuscular haemoglobin concentration

The mean cell haemoglobin concentration varies very little in normal pregnancy or when iron is given (Lund, 1951; Hytten & Duncan, 1956). There is, however, a definite decrease in iron deficiency anaemia. Vogel et al (1963) found the MCHC a valuable means for assessment of the degree of anaemia and also the response to treatment. Paintin & Hytten considered a fall in MCHC to be a rather late sign of iron deficiency. In the present work the mean value in the Cw group is low only in comparison with the mean values of the other groups but the difference at 36 weeks is significant. In addition at 36 weeks individual readings fall in a lower range.

Iron Metabolism

Iron balance in any individual depends on the needs of the body, the amount available in the diet, the efficiency of absorption and the daily loss in urine, faeces, sweat, desquamation of epithelial surfaces and, in women, menstruation. In men and non-pregnant women it is only necessary to replace iron lost from the body. Garby (1966) estimates this as 0.4 mgm/day in men and 1.0-2.0 mgm/day in women. He does not, however, include loss by desquamation in his calculation.

In pregnancy there is the additional burden of supplying iron to the foetus and placenta as well as to the increased red cell mass made necessary by the increased vascular bed. There are various estimates in the literature of the amount of iron needed by the pregnant woman. Scott (1962) quotes a final deficit of 680 mg if the woman breast feeds and 500 mg if she does not. Paintin et al (1966) calculate that by the end of pregnancy a total of 867 mg will be required. They do not estimate the amount of iron which will return to stores when the red cell mass returns to normal after delivery. Bothwell & Finch (1962) quote a total need, in pregnancy, of 550 mgm of iron in comparison with a requirement of 336 mg in a non-pregnant woman during the same period.

The need for iron varies throughout pregnancy. Bothwell and Finch (1962) consider that requirements in the first trimester are covered by the saving of iron due to amenorrhoea. The daily need in the first trimester given by Paintin et al (1966) is 2.1 mg. which is of the same order as the upper limit of iron required by a non-pregnant woman (Garby 1966). In the second trimester

a daily absorption of 3.9 mg. is needed and in the third trimester 4.2 mg. (Paintin et al 1966). These figures are very similar to those quoted by Bothwell and Finch (1962) when it is remembered that they do not allow for loss due to desquamation in their calculations. A survey in 1954 showed that the average diet in the United Kingdom contained 13 mg. of iron per day (Ministry of Agriculture, Fisheries and Food, 1956). It seems improbable that this has altered to any extent in recent years. The amount available for utilisation by the pregnant woman will depend on individual rates of absorption.

The most extensive work on the rate of iron absorption in pregnancy seems to be that of Hahn et al (1951). Using a dosage of 18 mg. of iron they demonstrated a median uptake of 10% in early pregnancy (<15 weeks) and this increased to 26% in the last four weeks of pregnancy. When therapeutic doses were given these figures fell to 2.2% and 8% respectively so that for a 6-7 fold increase in dose there was only a 2 fold increase in absorption. The authors comment on a wide individual variation in their results so these were expressed as "Median % Uptake" when "Uptake" = Factor of absorption + Utilisation. Prtichard and Adams (1960) also found evidence of increased red cell utilisation of iron. In their series, by the 7th day after injection, pregnant women showed 91% utilisation of the injected isotope in red cells as compared with a 76% utilisation in non-pregnant women.

been

Various factors have shown to influence absorption. Pirzio-Biroli & Finch (1960) found that the extent of the iron stores had a considerable

influence on the rate of absorption. A normal mean absorption of 6% was reduced to 3% when iron stores were increased to 1.5 g. Conversely absorption increased to 20% following sufficient reduction of iron stores.

Iron stores can most conveniently be estimated by examination of bone marrow. Conrad et al (1962) found that when young men were bled serially bone marrow was the last iron reserve to be depleted, and the last to recover.

Absence of marrow iron has been repeatedly observed in a high proportion of pregnant women (Holly & Grund, 1959; Allaire & Campagna, 1961). This finding has usually been related to iron deficiency but it seems possible that iron requirements in early pregnancy are to a large extent provided from marrow stores and reduction of these stores would then stimulate increased absorption. The work of Hahn and his colleagues provides some support for this possibility as they found a significantly higher uptake of iron in early pregnancy in the higher parity groups. Iron stores are likely to be reduced at the beginning of pregnancy in multiparous women (Scott 1961).

Iron is present in small quantities in the blood serum as well as in haemoglobin and in the body stores. Abderhalden in 1898 was the first to recognise the presence of iron in the blood which was not bound up in the haemoglobin molecule. Häuserman (1899) showed that this non-haemoglobin iron was present in the plasma. The work was extended and confirmed by later workers (Fontes & Thivolle, 1925; Warburg & Krebs 1927) and today this fraction is known as 'serum iron' (Dahl, 1948). More recently it has been demonstrated that iron is carried in serum bound to the protein fraction now identified as β -globulin (Surgenor et al 1949; Cartright & Wintrobe, 1949).

This is not a single entity, as several distinct bands can be distinguished, but as the ability to transport iron seems uniform, the complex is usually considered as such (Bothwell & Finch, 1962). The iron combining globulin is called transferrin and a proportion is always unsaturated. The level of unsaturated transferrin varies in relation to iron metabolism (Ventura & Klopfer, 1951).

The relationship of bound iron in the serum (serum iron) to total iron binding capacity (serum iron + unsaturated transferrin) can be expressed as % saturation of transferrin. Taylor & Gatenby (1966) found a significant relationship between this figure and the rate of iron absorption. There was also a significant inverse correlation with serum iron levels. Serum iron levels fall in normal pregnancy when no treatment is given (Ventura & Klopfer 1951; Holly, 1953). Rath et al (1950) are the only observers who report a maintenance of serum iron levels in late pregnancy and it has already been suggested that their patients may have taken iron (Hyttén & Duncan 1956).

A fall in serum iron levels will automatically cause a fall in % saturation of transferrin and this is further exaggerated by the increase in transferrin levels which occurs in pregnancy. Raised transferrin levels are also found in the presence of iron deficiency anaemia and the occurrence of this change in pregnancy has been put forward as an indication of iron deficiency, but raised levels persist even when iron is given.

Increased transferrin levels in pregnancy in the absence of iron deficiency may be explained by the work of Weinfeld (1965). This worker found that a rise in storage iron was associated with a fall in total iron binding capacity. It seems reasonable to deduce that utilisation of storage iron in

pregnancy could be associated with an increased iron binding capacity.

The fall in serum iron levels may have a hormonal basis.

Palmer (1952) found that non-menstruating women of menstrual age have the same lower serum iron levels that are found in menstruating women. A similar observation was made in rabbits who do not menstruate. In addition when oestrogens were given to both male and female rabbits there was a fall in serum iron. Fujino et al (1966) found an inverse correlation between serum iron and oestrogen levels but Zilva and Patston (1966) reported some correlation between low serum iron levels and the lowest levels of urinary oestrogen in normal menstrual cycles.

Active erythropoeisis also increases absorption of iron (Weintraub et al, 1965), and can indeed continue to promote iron absorption in the presence of high iron stores (Sephton Smith, 1965). It is a matter of general experience that marrow hyperplasia occurs in pregnancy (Callender 1946). Holly & Grund (1959) however, found evidence of depressed erythropoeisis in pregnancy. This was a very unexpected finding and the work was repeated by Pritchard and Adams (1960) and they found that erythropoeisis was actually accelerated.

It would therefore seem that in pregnancy there are physiological changes which promote increased absorption and utilisation of iron. In a normal woman with adequate iron stores and a sufficient diet it is unnecessary to give additional iron. Indeed, by artificially raising the serum iron one would seem to risk interference with the normal mechanism of the body.

It also has to be remembered that at the end of pregnancy the red cell volume is reduced to normal. Some blood is lost at delivery and in the placenta but there is still an appreciable volume of red cells to be broken down and

and the iron content will then be returned to storage. This 'pay-back' of iron has been variously estimated. Rath et al (1950) give a figure of at least 500 mg. Bothwell & Finch (1962) 100-140 mg. and Beaton (1966) 230 mg. If therefore, it is possible for a pregnant woman to continue to absorb iron in excess of her needs, large doses of mineral iron during pregnancy could lead to iron over load in the puerperium. This has been shown to occur in the Bantu women described by Gerritson & Walker (1954) as half of these women had haemochromatosis at autopsy (Hyttén & Duncan, 1956). These women are, however, a special group and in general large deposits of haemosiderin are rare in women (Finch & Finch, 1955).

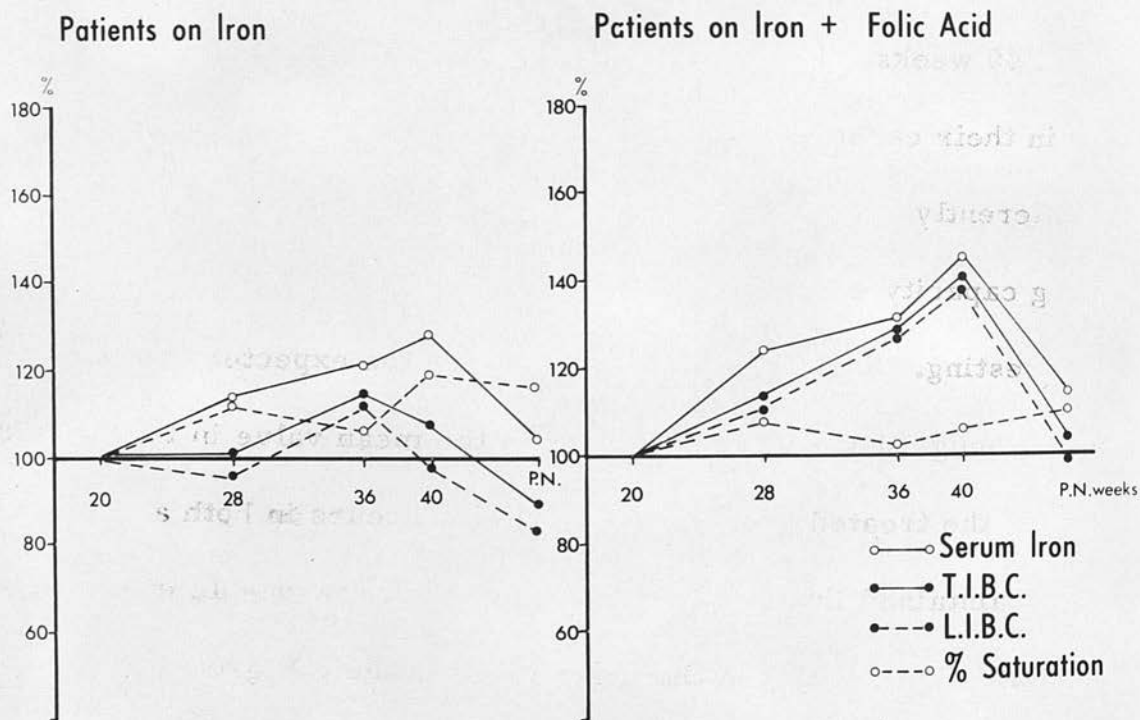
The present work is illustrated by scattergrams depicting the individual readings of serum iron, total iron binding capacity, latent (or unsaturated) iron binding capacity and % saturation. In these figures the mean values at the weeks studied are joined by a solid line (Figures 2-5). In addition an attempt has been made to illustrate the different patterns of iron metabolism in treated and untreated women. In these figures (Figures 6 & 7) the 20 week levels are taken as 100% and later readings are plotted as percentage increase or decrease on these values. Serum iron, total iron binding capacity, latent iron binding capacity and % saturation are plotted on one graph for each group studied. It is not suggested that these graphs express more than a general pattern of the behaviour of the variables as there is not complete uniformity between the 20 week levels in the different groups. This particularly applies to the Cu and Cw groups as these are sub-divisions of the original untreated group.

Mean serum iron levels rise during pregnancy in the treated groups. This has also been the experience of many previous workers (Sturgeon, 1959; Holly & Grund, 1959). The mean values and range are also similar. The experience of Verloop et al (1959) differed in that their patients treated with iron showed a slight fall in mean serum iron levels in late pregnancy. Malkosian et al (1964) after an initial rise on iron record a slight fall at 40 weeks. A wide range of individual readings seem to be a general finding and may explain lack of uniformity in mean values.

The untreated groups show the expected fall in serum iron levels. The pattern of the fall is not so steep in the Cw group but the 20 week level was significantly lower than in the other groups. The Cu group shows a maximum fall at 36 weeks when it is 27% below the 20 week level. This fall is of the same order as has been previously reported (Ventura & Klopper, 1951; Lund, 1951). At 40 weeks there is an unexpected rise in the mean value which is accompanied by a fall in unsaturated iron binding capacity; the total iron binding capacity continues to rise at this time. It therefore seems unlikely that this finding is associated with the fall in plasma volume which usually occurs in late pregnancy. The observation is difficult to explain but as only five readings are available at 40 weeks it would probably be unwise to attempt any interpretation of the finding until further studies are made.

The mean values for total iron binding capacity (TIBC) and latent iron binding capacity (LIBC) are rather lower in this investigation than have been previously reported. This may be related to the variety of methods used by different observers. Total iron binding capacity was the only variable in this study which was not comparable in the three main groups of the original

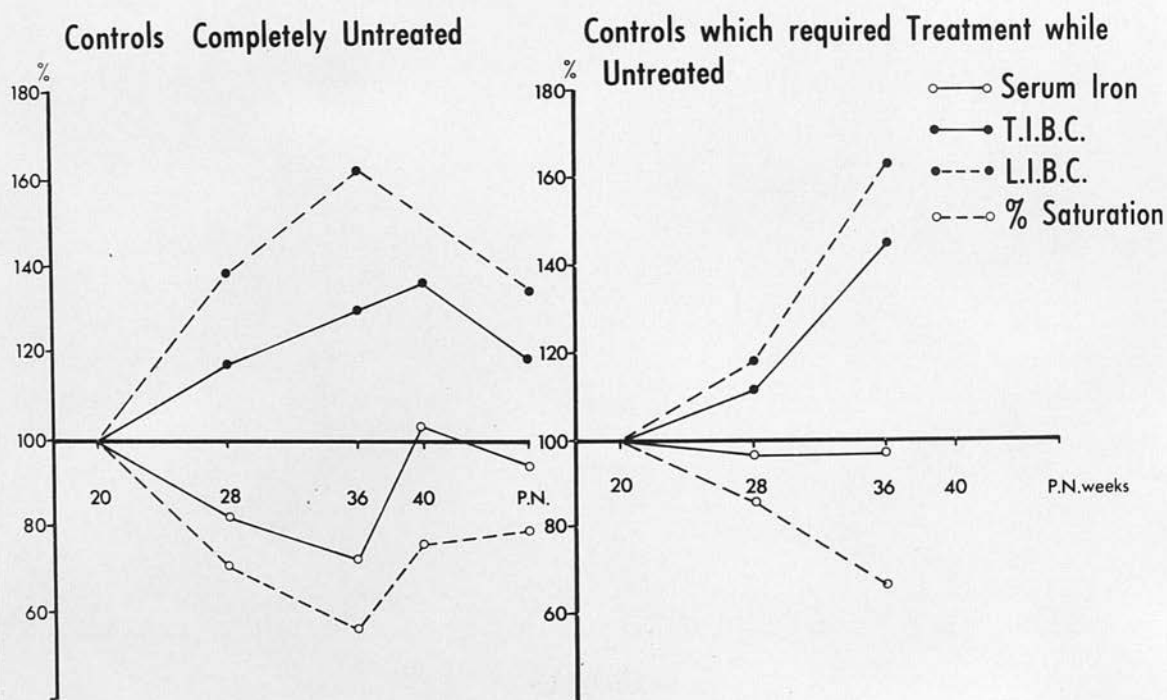
Fig. 6. Patterns of Iron Metabolism



protocol at 20 weeks. This seemed to be due to six wild readings in groups 1 and 2 (Vol. 2, p. 44-45). This view is supported by the almost identical mean value for TIBC which is seen in the group on iron at 28 weeks and the associated fall in the mean value of LIBC. With this exception TIBC and LIBC rise very similarly in the I, I+F and Cu groups until 36 weeks. Group Cw, however, shows a significant increase in the mean values of both TIBC and LIBC at 36 weeks. The group on iron demonstrates a fall in both of these variables at 40 weeks. Malkosian and his co-workers also record this behaviour in their cases treated with iron. The group on iron + folic acid behaved differently. In this group the total iron binding capacity and latent iron binding capacity continue to rise at 40 weeks. The behaviour of % saturation is also interesting. The untreated groups show the expected steep fall during pregnancy although there is a slight rise in the mean value in the Cu group at 40 weeks. In the treated groups an initial rise occurs in both and the level reached is maintained throughout pregnancy, with reasonable uniformity, in the group on iron. In comparison the mean value in the I+F group shows a fall at 36 and 40 weeks. It is suggested that this is a reflection of the relatively greater increase of the transferrin pool in women who receive folic acid in addition to iron.

The different modes of behaviour are shown more clearly in the graphs which are constructed by using % variation on 20 week levels for the four variables studied in each group. The increase of the transferrin pool is relatively greater in the untreated groups as judged by the patterns for total and latent iron binding capacity. Ventura & Klopper (1951) attributed this to

Fig. 7. Patterns of Iron Metabolism

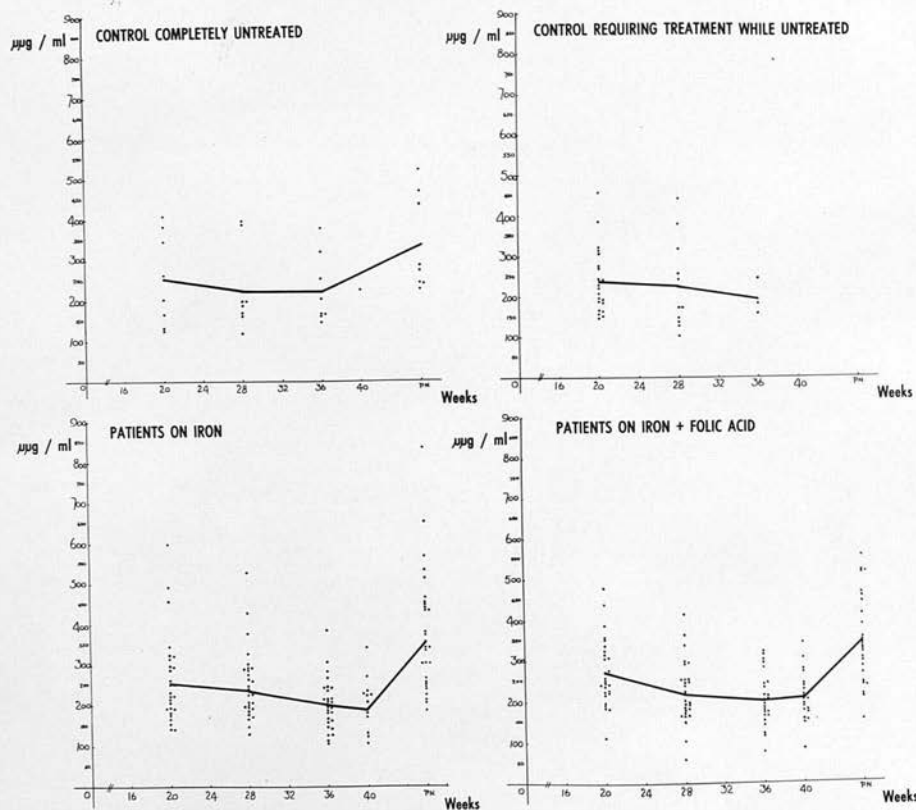


"the vastly increased turnover of iron in late pregnancy" but Hytten & Duncan (1956) thought it could be explained by the rise in concentration of plasma globulin. In the Cw group it seems likely that it also reflects some degree of iron deficiency. The treated groups also show an increased transferrin pool and this is relatively greater in the group on iron and folic acid. Lane (1966) found a close association between % saturation and total iron binding capacity. He also comments on the possibility that iron has some effect on liver activity in the synthesis of transferrin. It is generally accepted that folic acid plays an important part as folate co-enzyme in normal erythropoeisis and nuclear synthesis (Luhby & Cooperman, 1964) but otherwise little is known of its function in body metabolism. The present findings would seem to indicate increased activity of the transferrin pool when folic acid is given in addition to iron. It seems possible that folic acid may have some function in iron metabolism which is demonstrated when additional folic acid is given as well as additional iron. It is also suggested that therapeutic doses of mineral iron in normal pregnant women cause an imbalance of the metabolic process. The addition of folic acid would restore this balance and metabolic behaviour would then approximate more closely to that found in normal untreated women. Indeed, allowing for the differences due to increased serum iron the pattern of behaviour in the Cu group shows more similarity to the I+F group than to the group on iron only.

These observations, of course, are not intended to apply to women who are frankly deficient in either substance.

Fig. 8. Serum B₁₂ Levels; Scattergrams and Mean Values

SERUM B₁₂ LEVELS



Chapter 4

Observations: II. Serum B₁₂ and Serum folateSerum B₁₂. (Vol. 2, p. 65 and Figure 8).

Mean Values and Range ($\mu\text{g/ml}$)
(Number of estimations in brackets)

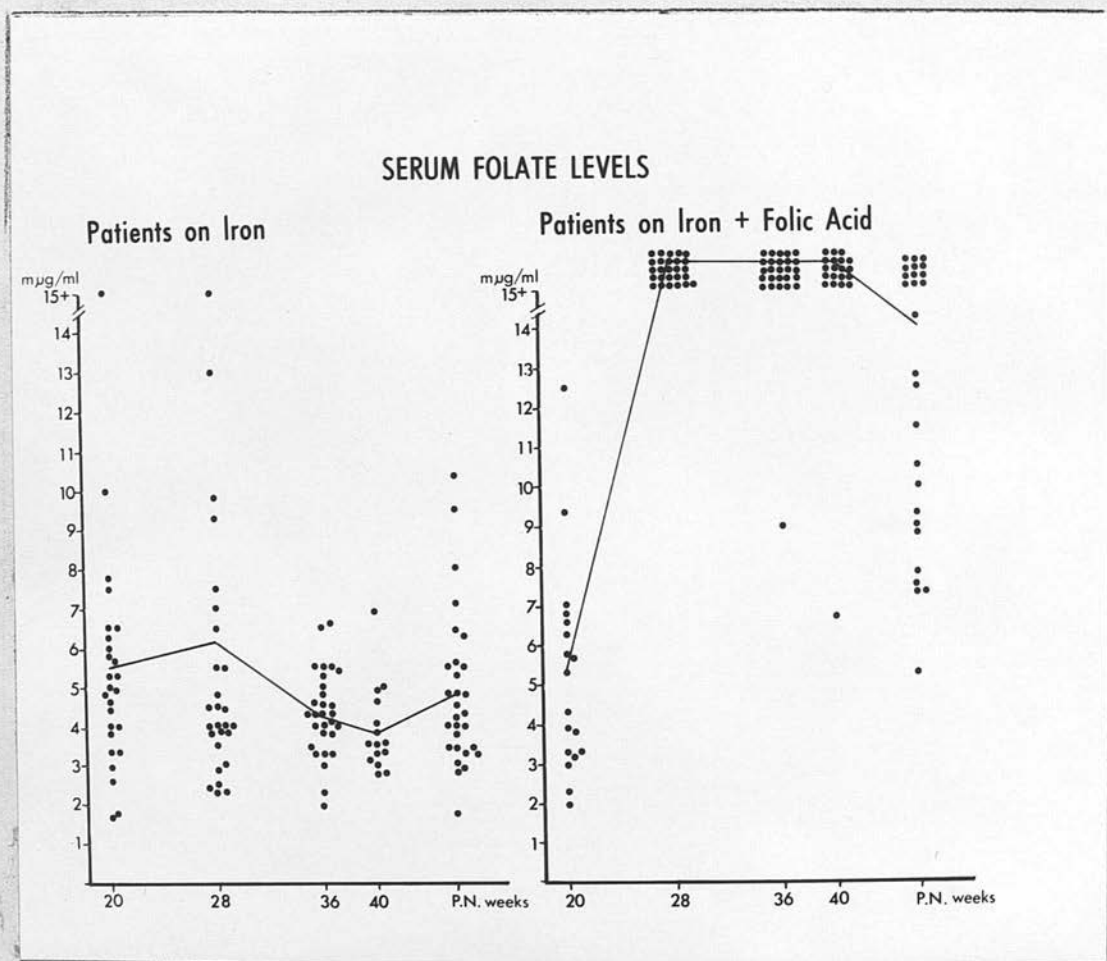
Weeks	I	I + F	Cu	Cw
20	268 (27) 140-598	273 (27) 115-479	254 (8) 128-409	239 (21) 152-459
28	252 (28) 130-528	222 (27) 57-412	227 (8) 120-397	227 (11) 104-444
36	212 (29) 105-504	204 (24) 73-320	224 (8) 145-379	195 (4) 159-246
40	202 (12) 108-341	208 (20) 83-341	- (1)	-
PN	375 (29) 189-835	348 (26) 153-556	337 (8) 228-520	-

There were no significant differences between any of the mean values at the weeks studied. Following the commencement of treatment 152 results were available for inspection in the group on Iron, 150 in the group on Iron and Folic Acid, 41 in the completely untreated group and 44 in the group which would require treatment. The number of readings of less than 100 $\mu\text{gms/ml}$ were counted in these groups with the following results.

Group	No. with Readings Below 100 $\mu\text{g/ml}$	%
I	2	0.013
I + F	4	0.027
CU	0	0
Cw	1	0.023

It was also observed on inspection that although the mean values

Fig. 9. Serum Folate; Scattergrams and Mean Values



at the end of pregnancy were very similar in both of the treated groups the initial rate of fall seemed to be much greater in the group on Iron + Folic acid. The proper correction for serial correlation was applied and mean values for the rate of fall in the treated groups were calculated for the periods, 20-28 weeks, 20-36 weeks and 20-40 weeks. When these were compared it was found that the rate of fall in the I + F group in the 20 - 28 week period was greater than the rate of fall in the group on Iron ($p < 0.05$). No significant differences were found between the groups for the other periods tested.

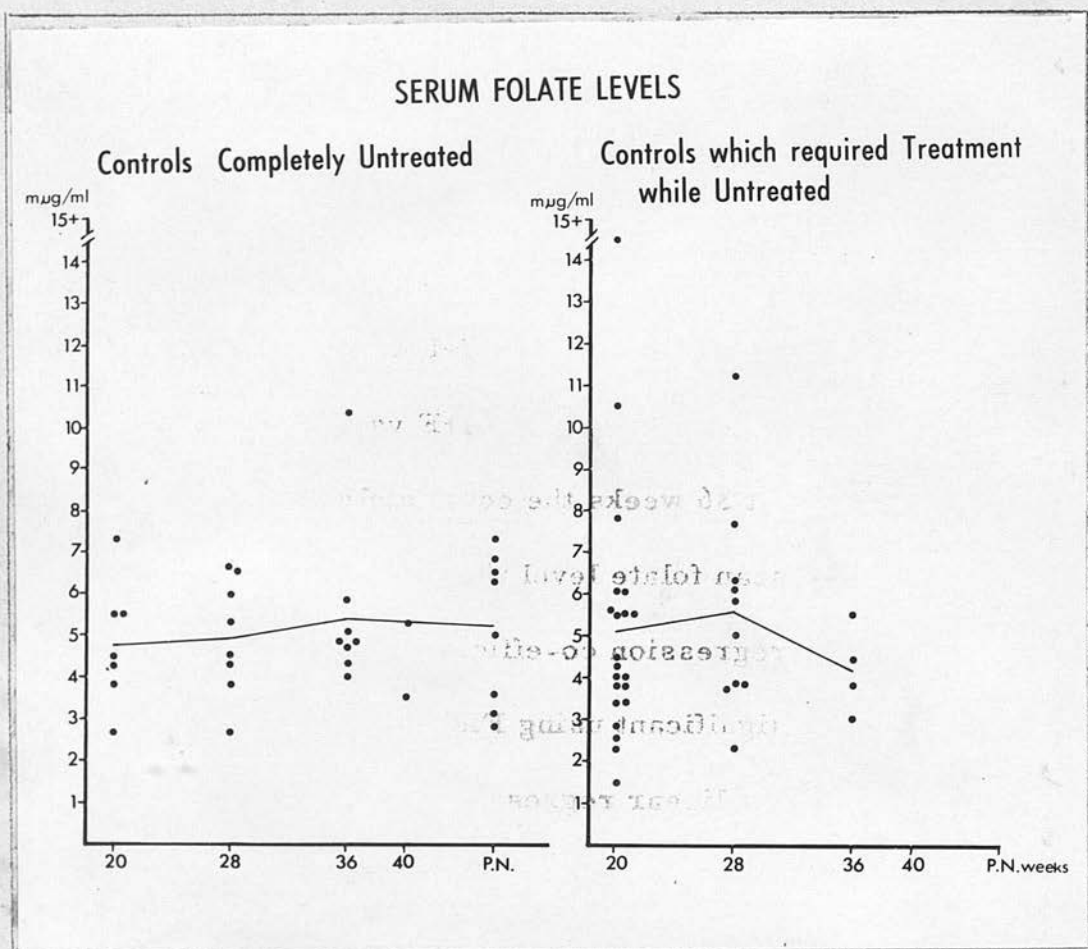
Serum folate. (Vol. 2, p. 72 and Figures 9 & 10)

Weeks	<u>Mean Values and Range (mg/ml.)</u> (Number of estimations in brackets)			
	I	I+F	Cu	Cw
20	5.5 (26) 1.7-20.5	5.3 (17) 2.0-12.5	4.7 (7) 2.7-7.3	5.1 (21) 1.5-14.5
28	4.9* (26) 2.3-13.0	54.3 (26) 18.3-70.0	4.9 (8) 2.6-6.6	5.6 (10) 2.3-11.2
36	4.3 (28) 2.0-6.6	51.7 (26) 9.0-70.0	5.4 (8) 4.3-10.3	4.2 (4) 3.0-5.5
40	3.8 (15) 2.8-6.9	54.2 (20) 6.7-70.0	- (2)	-
PN	4.8 (29) 1.8-10.3	14.0 (26) 5.3-34.0	5.2 (8) 2.8-7.3	-

* One result of 38 mg/ml was excluded in calculating the mean. ;

There were no significant differences between the mean values at 20 weeks. At the remaining weeks tested as would be expected, the mean serum folate levels in the I+F group were significantly higher than in the other groups:-

Fig. 10. Serum Folate; Scattergrams and Mean Values



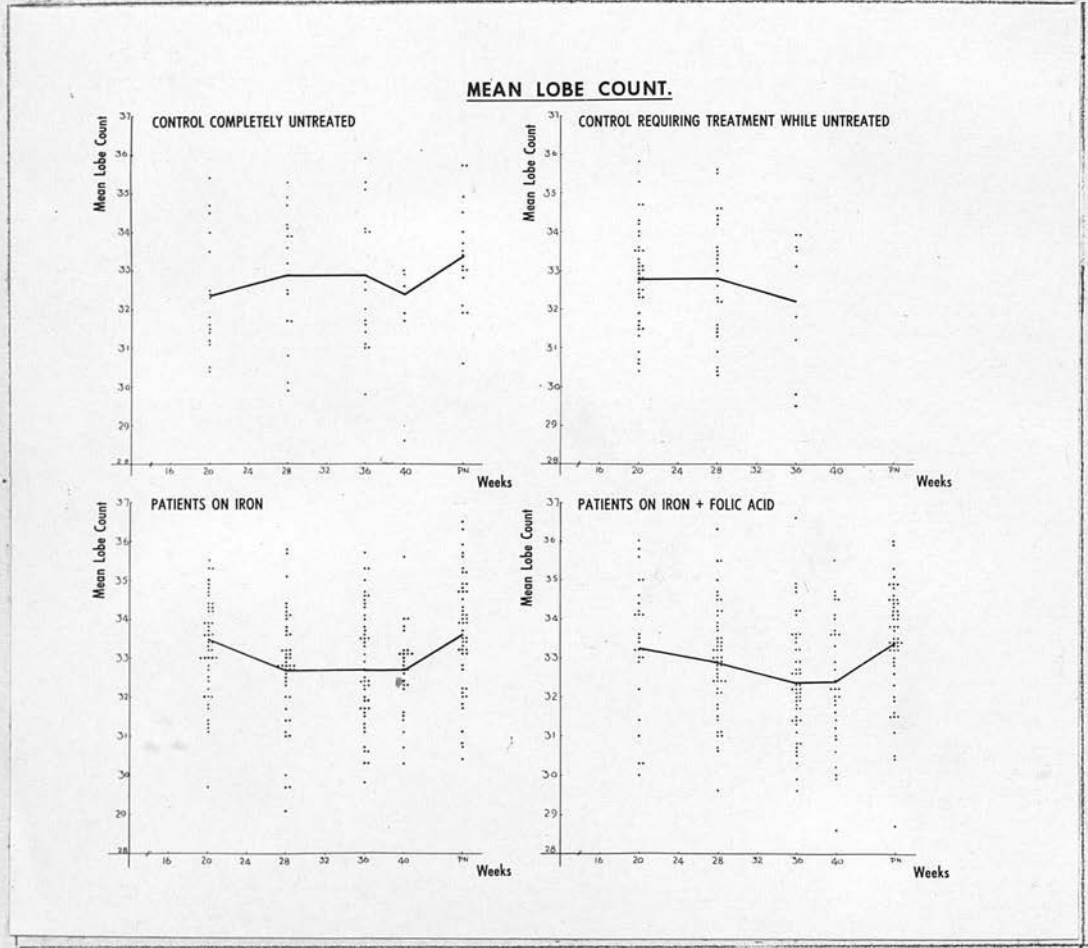
28 weeks	I+F vs I : p < 0.0005
	I+F vs Cu: p < 0.0005.
	I+F vs Cw: p < 0.0005
36 weeks	I+F vs I : p < 0.0005
	I+F vs Cu: p < 0.0005
	I+F vs Cw: p < 0.0005
40 weeks	I+F vs I : p < 0.0005
PN.	I+F vs I : p < 0.0005
	I+F vs Cu: p < 0.0005

In addition at 36 weeks the completely untreated group had a significantly higher mean folate level than the group on Iron ($p < 0.025$).

The mean regression co-efficient was negative in the group on iron and this was significant using Fisher's test ($p < 0.05$). The I+F group showed a positive linear regression and the mean regression co-efficient was significantly greater than in the other two groups ($p < 0.005$).

The wide range of readings in the I+F group is probably due to the relationship between the time of day at which specimens were taken and the time of taking tablets. Cases where relatively low figures were recorded during pregnancy had high readings at the post-natal visit so that failure to take tablets was not suspected.

Fig. 11. Mean Lobe Counts; Scattergrams and Mean Values



Mean lobe counts. (Vol. 2, p. 77 and Figure 11).

Mean Values and Range
(Number of estimations in brackets)

Weeks	I	I + F	Cu	Cw
20	3.34 (48) 2.97-3.78	3.33(26) 3.00-3.60	3.24 (16) 3.04-3.54	3.28 (37) 3.06-3.58
28	3.27 (46) 2.91-3.58	3.29 (51) 2.96-3.63	3.29 (16) 2.99-3.53	3.28 (25) 3.03-3.56
36	3.27 (47) 2.98-3.57	3.24 (49) 2.96-3.66	3.27 (16) 2.98-3.59	3.22 (9) 2.95-3.39
40	3.27 (27) 3.03-3.56	3.24 (32) 2.86-3.55	3.18 (6) 2.86-3.30	-
PN	3.36 (48) 3.04-3.65	3.34 (51) 2.87-3.60	3.34 (16) 3.06-3.57	-

The only significant differences between mean values were found at 20 weeks:

Cu vs I : $p < 0.05$

Cu vs I+F: $p < 0.01$

The mean regression co-efficient was positive in the completely untreated group and negative in both of the treated groups. These differences were significant:-

Cu vs I : $p < 0.05$

Cu vs I+F: $p < 0.01$

There was also a significant correlation between Serum Folate level and Mean lobe Count in the group on iron + folic acid ($r = -0.295$, $p < 0.01$). This correlation was not demonstrated in the other groups.

Discussion

Pernicious anaemia of pregnancy was recognised before the publication of Biermer's reports of 1868 and 1871. Many of Biermer's cases were associated with pregnancy and for some years there seems to have been some confusion in distinguishing between pernicious anaemia of pregnancy and primary pernicious anaemia, (Pepper, 1927). The situation was clarified by Osler (1919) when, in describing the severe anaemias of pregnancy and the post-partum period, he pointed out that "although progressive and often pernicious, the anaemia is caused by an agent which differs in one all important particular from that which causes the anaemia of Addison. When recovery takes place it is permanent, and the woman may escape in subsequent pregnancies".

The next important advance followed the work of Wills in India. She described severe macrocytic anaemia of pregnancy which responded to treatment with liver or marmite (Wills & Mehta, 1930). Similar anaemias in non-pregnant women and men were described, particularly in tropical countries (Moore et al, 1944). It is not always clear whether these are megaloblastic anaemias as the marrow biopsy rate was low. In 1945 Spies et al demonstrated that patients with pernicious anaemia and nutritional macrocytic anaemias responded to folic acid which was a member of the vitamin B complex. Megaloblastic anaemia of pregnancy also responded to folic acid (Moore et al, 1945). It was soon found that this substance was not the active anti-pernicious

anaemia factor of liver as there was no improvement in the neurological conditions associated with pernicious anaemia (Heinle & Welch 1947), and often the y progressed more rapidly (Ross et al, 1948). At about the same time Vitamin B₁₂ was isolated and was soon shown to be identical with Castle's extrinsic factor (Berk et al, 1948).

In addition to iron there were now two further substances which had been shown to be necessary for normal erythropoeisis. Vitamin B₁₂ was specific for the treatment of primary pernicious anaemia but, at least in temperate zones, megaloblastic anaemia of pregnancy, dietary deficiency and malabsorption responded to folic acid.

Folic acid deficiency anaemia of pregnancy has been reported with increasing frequency in recent years. Stevenson (1938) reported 30 cases in a 6 year survey, Thompson & Ungley (1951) found 46 cases in 17 years but at this time, Lund (1951) estimated that 1% of all pregnancy anaemias in the New Orleans area were of this type. Since then the incidence reported in the United Kingdom has risen markedly. Giles & Shuttleworth (1958) roused considerable interest in the condition when they reported an incidence of 2.8% in Stoke on Trent. By 1965 Hibbard et al described megaloblastic changes in the marrow in 4.2% of a group of 167 unselected women attending an ante-natal clinic in Liverpool. They concluded that not less than 10% of women in their area had folate deficiency during pregnancy and that in nearly half of the cases it was sufficiently severe to cause megaloblastic erythropoeisis.

The concept of folic acid deficiency without megaloblastosis of the marrow is of fairly recent origin. It has arisen with the recognition

of earlier changes in red and white cells and their precursors e.g. macro-ovalocytes, atypical normoblasts, transitional cells, giant metamyelocytes and hypersegmented neutrophils. Some workers also find excretion of formimino-glutamic acid (FIGLU) in the urine after histidine loading a useful test of folate deficiency (Hibbard et al 1965; Scott & Sommerville, 1965). Chanarin et al (1963), Chisolm & Sharp (1964) and Chanarin (1964) did not find this a dependable test in pregnancy because of alterations in histidine metabolism.

The classical paper on the pathogenesis of megaloblastic anaemia in pregnancy was published in 1959 by Chanarin, O'Sullivan, MacGibbon and Mollin. These workers studied the clearance of folic acid from the plasma after intra-venous injection of a small dose. The rate of clearance was found to be increased in two-thirds of women in late pregnancy and in all women with twin pregnancies. Clearance rates were even higher in women with megaloblastic anaemia of pregnancy. It was concluded that folic acid deficiency was due to foetal requirements exceeding the dietary intake with the additional possibility that impaired absorption might also play a part.

Lowenstein et al (1962) found good correlation between megaloblastic anaemia in pregnancy and serum folate activity when *L. casei* was used. They considered that in pregnancy values of 3.1 - 4.0 $\mu\text{g/ml}$ indicated borderline deficiency. Solomons et al (1962), however, reported a mean value of 4.3 $\mu\text{g/ml}$ in normal pregnancy as compared with a mean value of 7.8 $\mu\text{g/ml}$ in the non-pregnant.

Herbert (1964) discussed the value of the presence of hypersegment

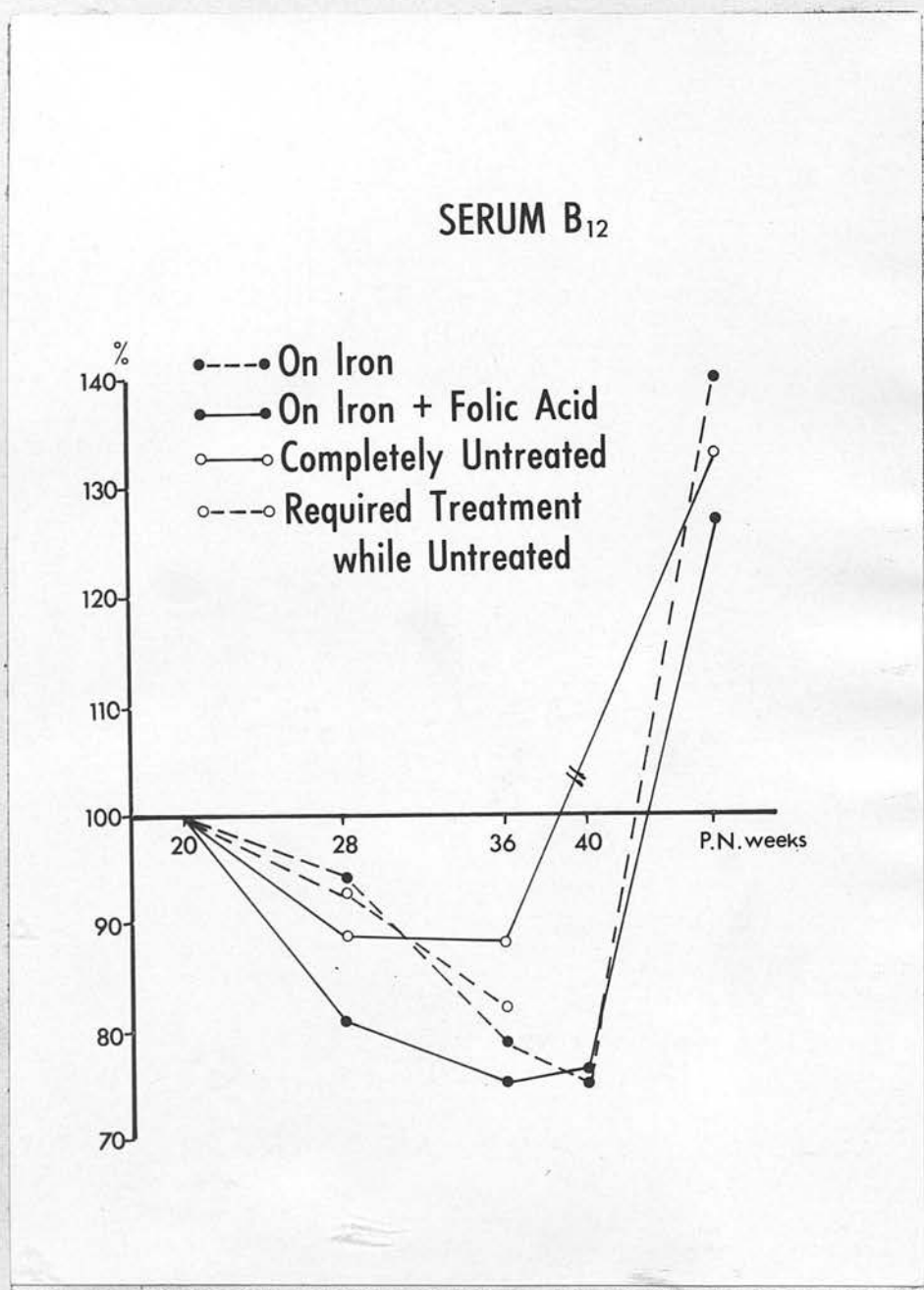
ed neutrophils (PMN) as a means of early diagnosis. He recommended making a mean lobe count per 100 PMN as a simple way of expressing the result. In his laboratory the normal mean lobe count was 3.17 (± 0.25) but the figure is liable to vary from one laboratory to another. For this reason Chanarin et al (1965) preferred to note the percentage of cells with 5 or more lobes. They gave 3% as the upper limit of normal.

In 1965 Chanarin and his colleagues published a comprehensive study of folic acid status in pregnancy. The construction of the project was similar to that described in the present work. Groups of women on iron, iron + 30 μ g. folic acid or on a placebo were followed through pregnancy and the whole question of folic acid status in pregnancy was discussed. They reached the conclusion that the diagnosis of megaloblastic anaemia in pregnancy and in the non-pregnant was still dependent on marrow morphology. Serum folate & Figlu estimations were not uniformly reliable. They found that even transient presence of hypersegmented neutrophils was a valuable indication that examination of the marrow was advisable. Megaloblastosis could, however, be present with no other indications of abnormality. Among the 15 cases with megaloblastic erythropoiesis 4 were found in women who had marrow biopsies done in order to obtain controls. They confirmed the observation of Solomons et al that serum folate levels fall during pregnancy and concluded that this indicated a high incidence of sub-clinical folic acid deficiency. They also found that serum folate levels were lower in the presence of iron deficiency and considered this condition to be an

important aetiological factor in the development of folic acid deficiency. This view was supported by Varadi (1965) and also by the work of Vitale et al (1966) on rats. These workers induced morphological changes of folate deficiency in rats kept on an iron deficient diet even when adequate folic acid was supplied. They concluded that this effect was related to the decreased activity of the enzyme formimino-transferase which depends on iron for optimal activity. Giles & Ball (1965) disagreed with this finding. They pointed out that it was a matter of clinical experience that folic acid deficiency could be precipitated when large doses of iron are given. These observations are not incompatible, as the behaviour of individual cases will depend on the relative sufficiency or deficiency of iron and folic acid.

Many workers now advise the routine administration of folic acid in pregnancy (Lowenstein et al, 1962; Giles, 1966); at least in industrial areas or where dietary deficiencies are common. There was some concern at first that routine treatment with folic acid could precipitate neurological complications in women with undetected pernicious anaemia. Witts (1962) pointed out that although pernicious anaemia can occur in the childbearing period it is usually associated with sterility so that pregnancy is very unlikely to occur. Although serum B₁₂ levels fall in normal pregnancy (Ball & Giles, 1964; Metz et al, 1965) it seems unlikely, even if there is a greater fall when additional folic acid is given, that this would be sufficient to precipitate pernicious anaemia as body stores of vitamin B₁₂ have been shown to last 4 or 5 years after total gastrectomy. This problem would seem to have

Figure 12. Pattern of Serum B₁₂ Changes



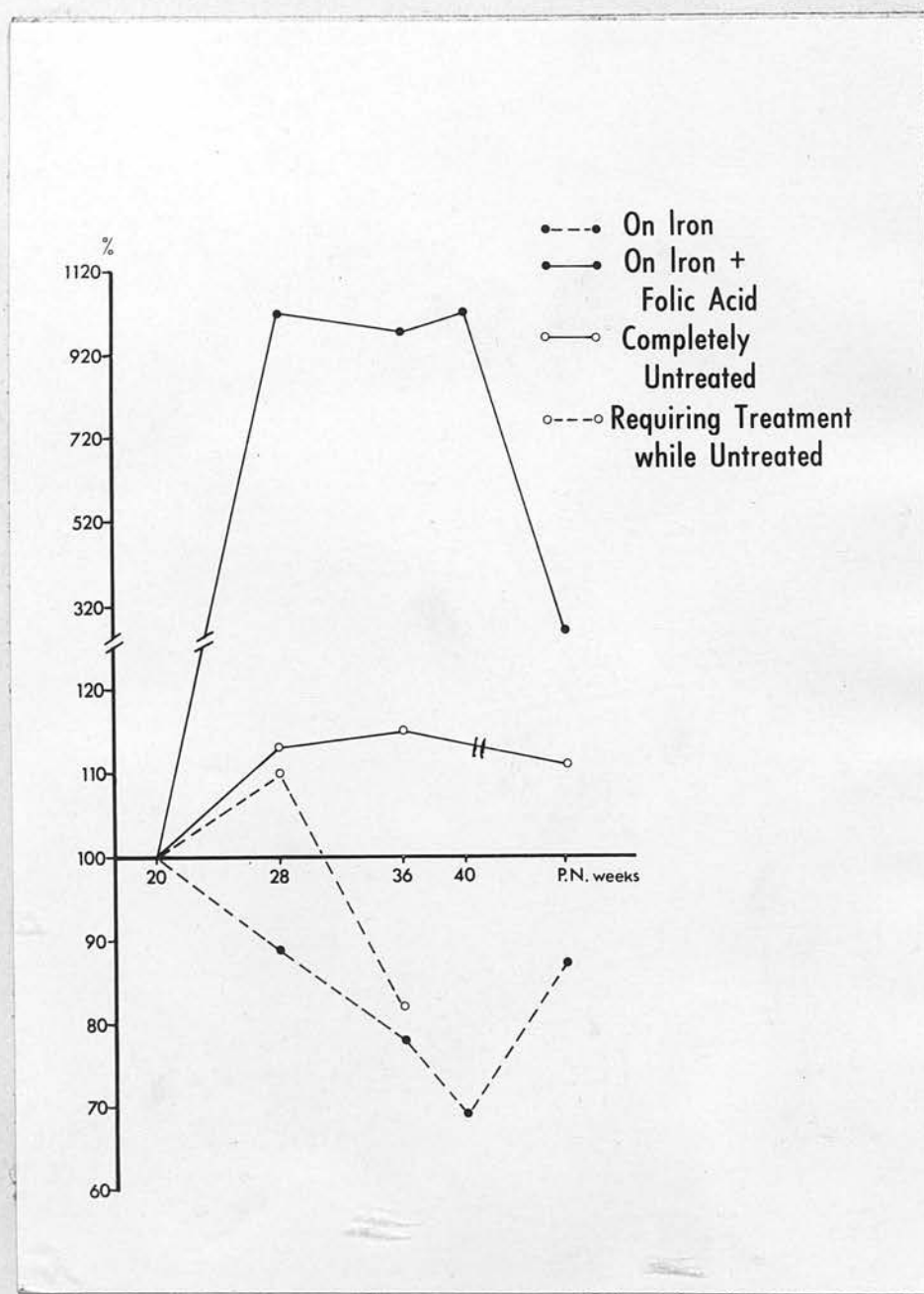
Note: The graph for the Cu group is broken at 40 weeks because of insufficient results.

been solved by the recent work of Willoughby & Jewell (1966) who found that when 300 μg of folic acid were given daily in pregnancy serum folate levels comparable with those found in normal adults were maintained. This is fortunate as Herbert (1966) has shown that daily doses of 400 μg of folic acid may produce a response in some cases of megaloblastic anaemia due to vitamin B₁₂ deficiency.

The results of the present work are illustrated by Figures 8, 9, 10, and 11 which show individual readings for serum B₁₂, serum folate and mean lobe counts presented as scattergrams with a line joining the mean values. In addition the patterns of behaviour of these three variables are again illustrated diagrammatically by expressing the readings as the percentage variation on the 20 week level which is taken as 100% (Fig. 12, 13, & 14).

A comparison of serum B₁₂ and folate levels in the four groups of women in this investigation would seem to show a relationship between the metabolism of iron, folic acid and Vitamin B₁₂. The completely untreated group as has already been said are haematologically well adjusted. In this group no imbalance is produced by giving additional haematinics. Mean serum B₁₂ levels fall in the Cu group at least to 36 weeks (unfortunately there was only one reading at 40 weeks). The fall in serum B₁₂ in pregnancy is generally reported and also the rapid return to normal levels which occur after delivery. Metz et al (1965) suggest that the fall represents a change in metabolism due to pregnancy and that the rapid rise after delivery expresses the removal of whatever factor has caused the depression. There is no rise in

Fig. 13. Pattern of Serum folate changes.



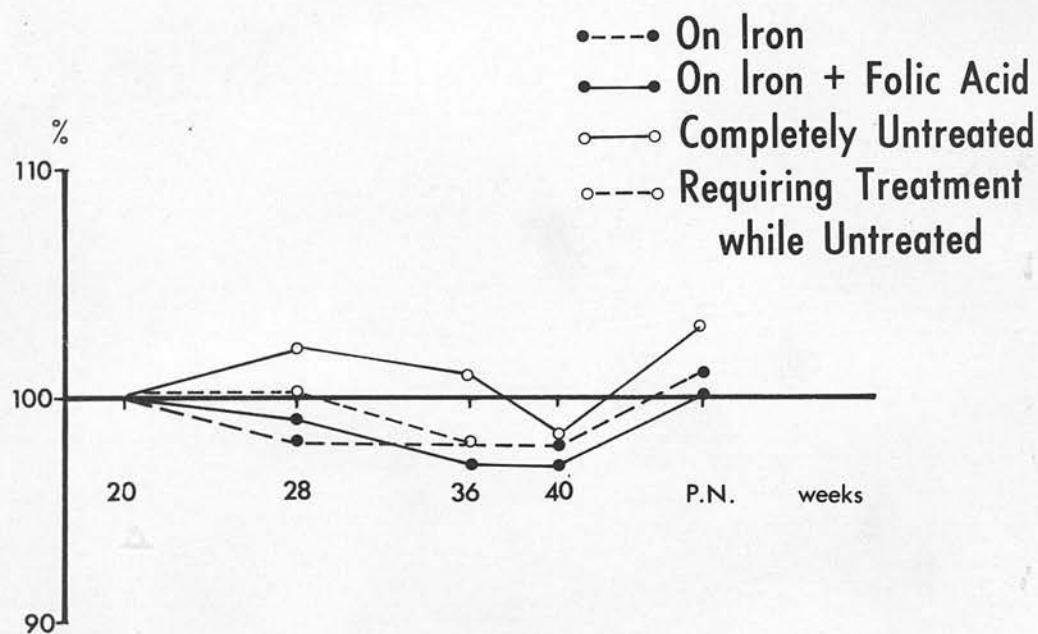
Note:- The graph for the Cu group is broken at 40 weeks because of insufficient results.

late pregnancy so there would not seem to be any association with volume changes. The fall by 36 weeks is greater in the treated groups, both when the mean values are studied and also when the percentage fall on the 20 week level is considered. It is of interest that the Cu group and the group on iron + folic acid show the greatest rate of fall between 20 and 28 weeks. The rate of fall then flattens so that similar levels are reached by the group on iron and the I+F group at 36 and 40 weeks. It is suggested that normal behaviour is represented by the Cu group while the treated groups respectively reflect lack of folic acid and vitamin B₁₂ or of vitamin B₁₂. It would be of interest to make a similar study when additional vitamin B₁₂ is also given. Metz and his colleagues report a similar pattern when 50 µg vitamin B₁₂ were given daily but they mention a transient rise of mean values in the 8 week period after starting the supplements.

The serumfolate levels are of particular interest. There was no fall in the mean folate levels in the Cu group. This would seem to be a contradiction of the findings of Chanarin et al (1965) but this is not necessarily so. The untreated group of these workers was comparable with the Cu and Cw groups of the present investigation and there is a relatively rapid fall of the mean serumfolate value in the Cw group at 36 weeks. In the same way the serum B₁₂ levels show the lowest mean value recorded in the investigation at 36 weeks in this group. The range of both variables is also lower. In contrast to the normal untreated group women on iron show a gradual fall in mean serum folate levels throughout.

Fig. 14. Mean Lobe Counts; Pattern of Behaviour

MEAN LOBE COUNT



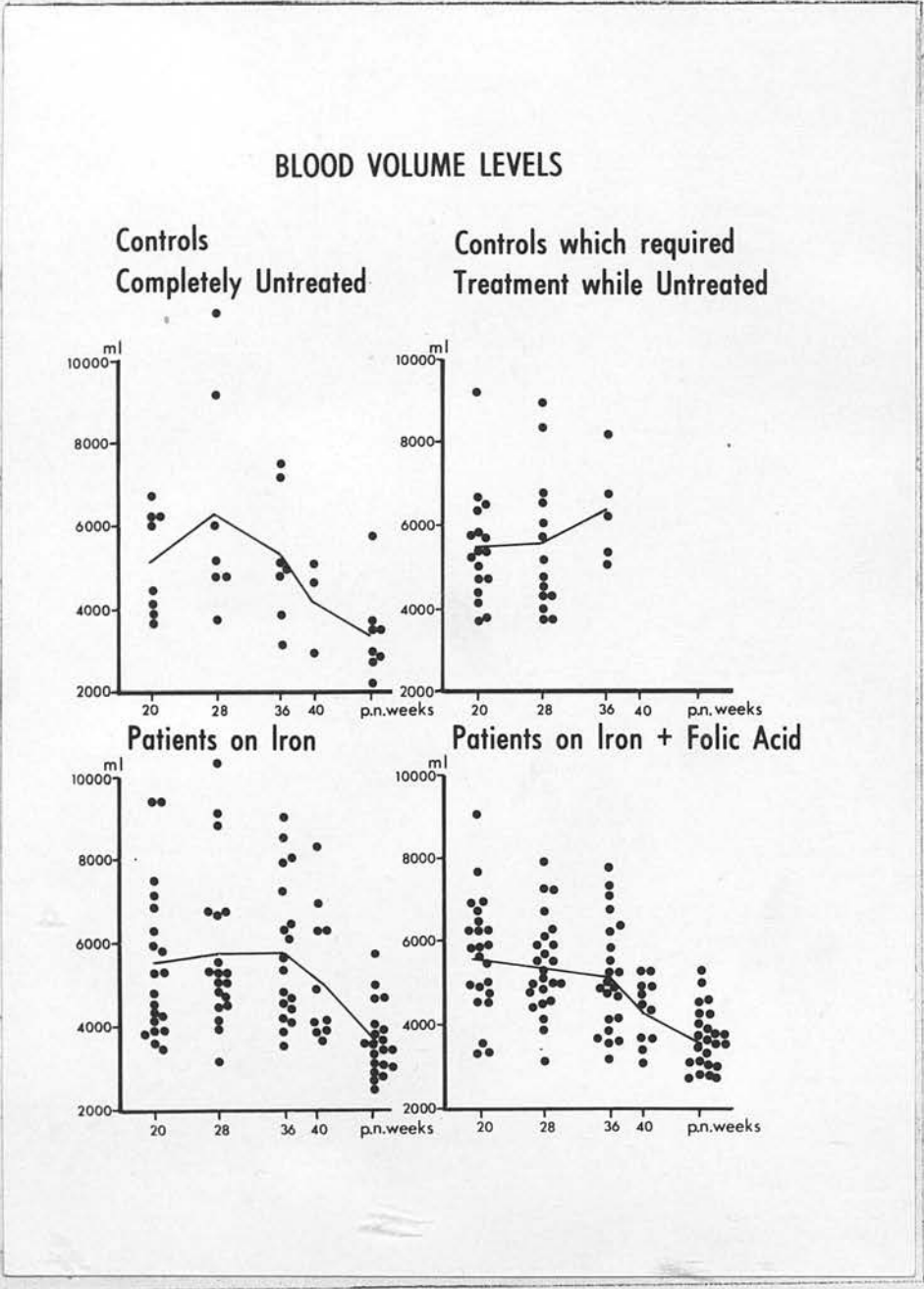
pregnancy with a return to a normal mean value at the post-natal visit. It is again suggested that this reflects an imbalance of iron and folic acid.

The mean lobe count in the Cu group was significantly lower than the mean lobe counts in the treated groups at 20 weeks so that comparison of the changes is approximate. The general pattern in normal pregnancy would seem to be that there is no alteration from the non-pregnant state. A fall is noted at 40 weeks but only 4 values are represented.

Both of the treated groups show a fall in the mean lobe count throughout pregnancy with a return to the 20 week level at the post-natal visit; by which time no supplements had been given for 6-8 weeks. The fall in mean lobe count in the group on iron is of interest in view of the finding of Chanarin and others that hypersegmented neutrophils can be associated with iron deficiency anaemia as well as with megaloblastic anaemia (Chanarin et al, 1965). This point is also illustrated by the case of Mrs. J. M. in the present series (Vol. 2, p. 165).

Hypersegmented neutrophils were rarely seen, but it is of interest to note that at the post-natal visit 3 of the 46 women on iron had blood films with 3% hypersegmented neutrophils (5^+ lobes) while in the I+F group only one of the 50 women examined reached this figure.

Fig. 15. Blood volume; Scattergrams and Mean Values



Chapter 5

Observations: III. Volume Changes.

Blood Volume . (Vol. 2, p. 85 and Figure 15).

Mean Values and Range (ml)
(Number of estimations in brackets)

Weeks	I	I + F	Cu	Cw
20	5489 (20) 3500-9400	5640 (23) 3240-9150	5160 (8) 3695-6770	5406 (17) 3620-9200
28	5760 (19) 3120-10300	5408 (24) 3160-7960	6360 (7) 3620-11100	5442 (14) 3600-8940
36	5780 (18) 3530-9050	4140 (22) 3120-7710	5270 (8) 3200-7460	6268 (5) 5040-8100
40	5200 (12) 3690-8310	4310 (11) 3030-5270	4190 (3) 2930-5010	-
PN	3624 (20) 2590-5640	3660 (24) 2770-5290	3367 (8) 2200-5750	-

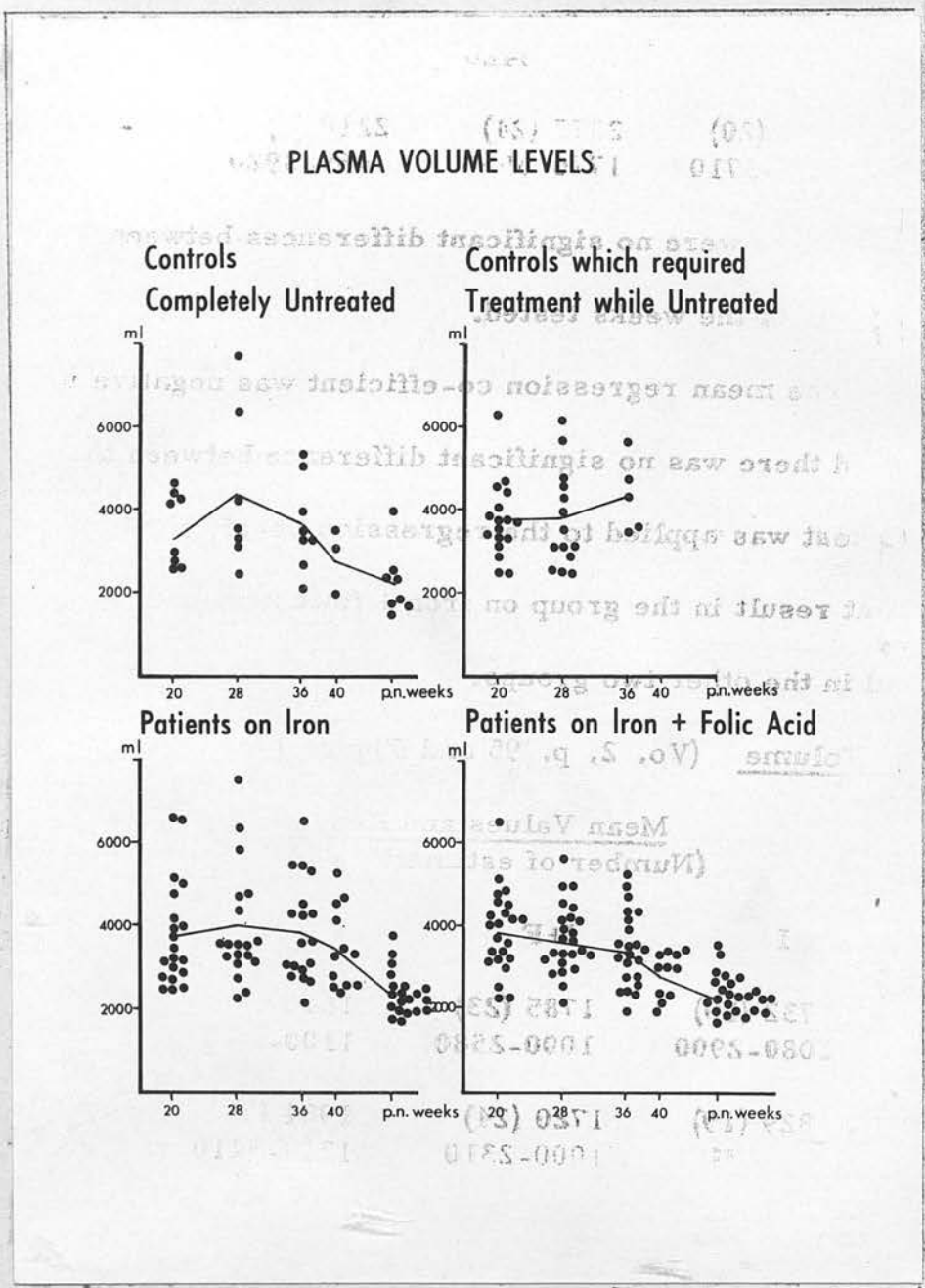
There were no significant differences between any of the groups at any of the weeks tested.

Plasma Volume. (Vol. 2, p. 90 and Fig. 16)

Mean Values and Range. (ml)
(Number of estimations in brackets) .

Weeks	I	I+F	Cu	Cw
20	3757 (20) 2420-6600	3856 (23) 2240-6570	3520 (8) 2595-4570	3728 (17) 2470-6250
28	3931 (19) 2220-7500	3685 (24) 2160-5650	4381 (7) 2410-7690	3763 (14) 2460-6190

Fig. 16. Plasma Volume; Scattergrams and Mean Values



Weeks	I	I+F	Cu	Cw
36	3857 (18) 2160-6500	3477 (22) 1940-5260	3610 (8) 2155-5300	4340 (5) 3480-5600
40	3439 (12) 2330-5240	2843 (11) 1910-3420	2776 (3) 1900-3420	-
PN	2355 (20) 1700-3710	2377 (24) 1770-3560	2218 (8) 1465-3920	-

There were no significant differences between any of the groups at any of the weeks tested.

The mean regression co-efficient was negative in the groups studied and there was no significant difference between them. When Fisher's test was applied to the regression co-efficients there was a significant result in the group on iron + folic acid ($p < 0.05$). This was not found in the other two groups.

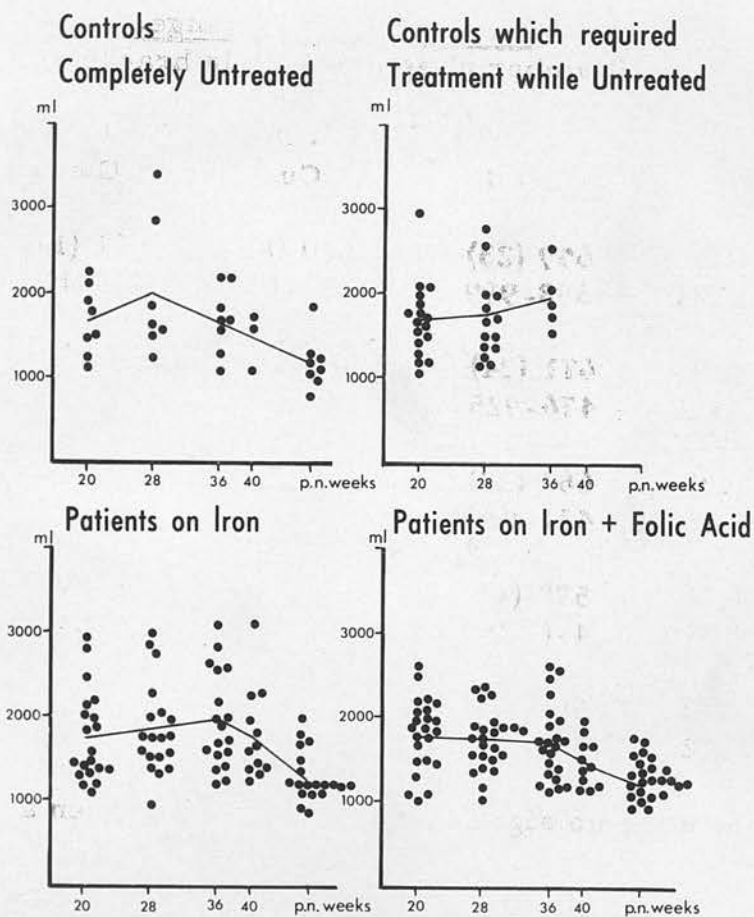
Red cell Volume (Vo. 2, p. 95 and Figure 17).

Mean Values and Range (ml)
(Number of estimations in brackets)

Weeks	I	I+F	Cu	Cw
20	1732 (20) 1080-2900	1785 (23) 1000-2580	1646 (8) 1100-2200	1678 (17) 1080-2950
28	1829 (19) 900-2940	1720 (24) 1000-2310	1984 (7) 1210-3410	1681 (14) 1140-2750
36	1925 (18) 1190-3070	1695 (22) 1120-2580	1659 (8) 1045-2170	1928 (5) 1560-2500
40	1765 (12) 1220-3070	1466 (11) 1120-1950	1420 (3) 1030-1640	-
PN	1269 (20) 850-1930	1285 (24) 910-1730	1149 (8) 735-1830	-

Fig. 17. Red Cell Volume; Scattergrams and Mean Values

RED CELL VOLUME LEVELS



There were no significant differences between any of the groups at any of the weeks tested.

The mean regression co-efficient was negative in the three groups tested and there was no significant difference between them. No significant result was found in any group when Fisher's test was applied.

Haemoglobin Mass. (Vo. 2, p. 100 and Figure 18).

Mean Values and Range. (G)
(Number of estimations in brackets)

Weeks	I	I + F	Cu	Cw
20	690 (20) 406-1184	699 (23) 398-999	629 (8) 453-855	661 (17) 445-1204
28	724 (19) 384-1110	671 (24) 476-925	774 (7) 471-1290	644 (14) 436-1080
36	750 (18) 449-1180	663 (22) 431-964	661 (8) 413-904	730 (5) 625-916
40	702 (12) 480-1230	578 (11) 440-780	553 (3) 405-633	-
PN	509 (20) 344-812	505 (24) 354-694	456 (8) 292-725	-

There were no significant differences between any of the groups at any of the weeks tested.

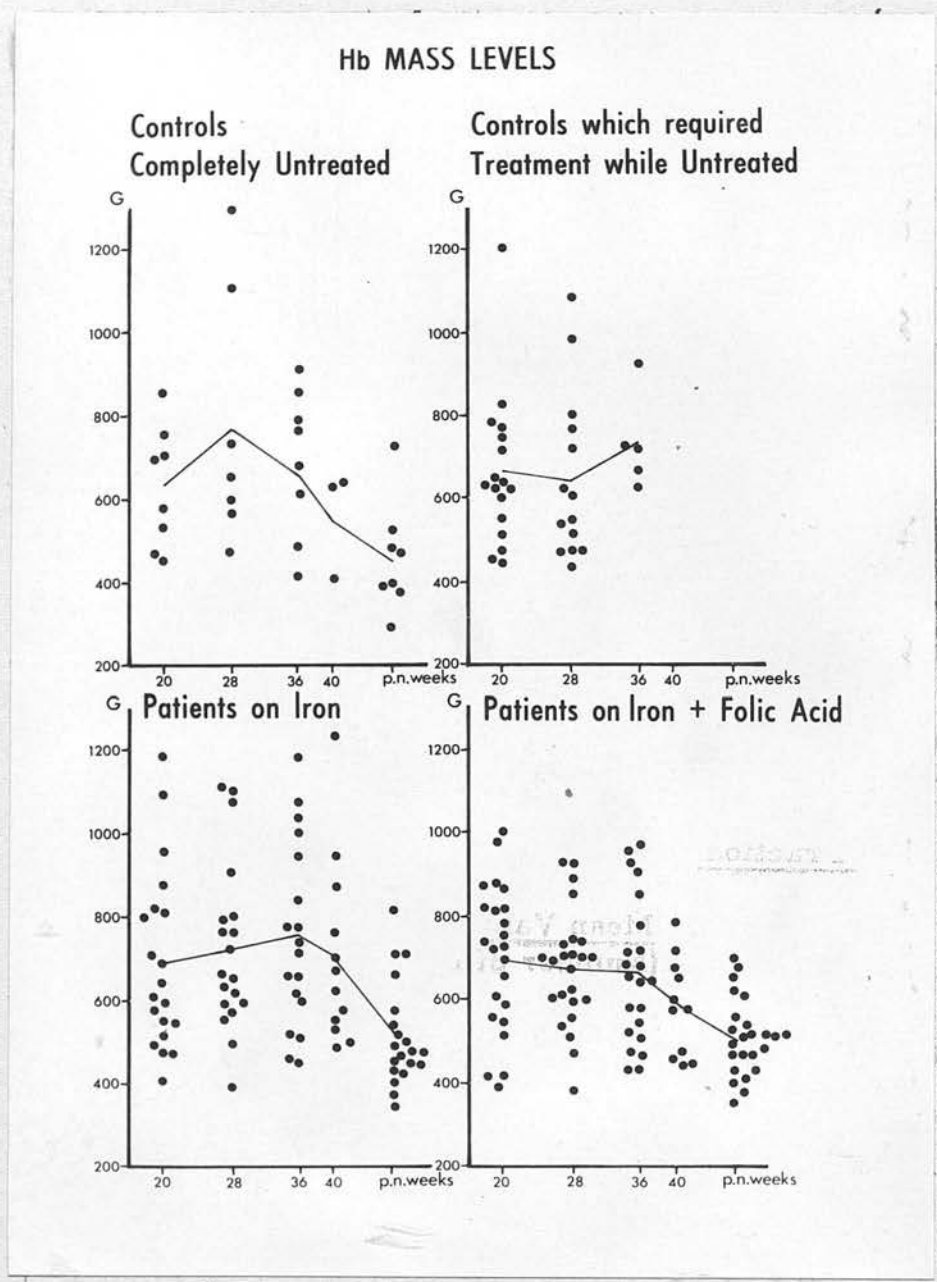
Protein Electrophoresis (Vol. 2, p. 145)

Albumin Fraction

Mean Values and Range (%)
(Number of estimations in brackets)

Weeks	I (16)	I+F (18)	Cu (4)
20	65.4 58.0-71.0	66.2 62.5-73.5	66.2 60.5-72.3

Fig. 18. Haemoglobin Mass; Scattergrams and Mean Values



Weeks	I (16)	I+F (18)	Cu (4)
36	62.8 54.5-68.5	63.1 56.2-69.6	62.2 56.8-69.1
PN	69.6 64.8-75.0	71.2 63.3-78.0	72.5 69.2-75.8

α_1 Globulin Fraction

Mean Values and Range (%)
(Number of estimations in brackets)

Weeks	I (16)	I+F (18)	Cu (4)
20	3.45 1.92-4.34	3.29 2.42-4.61	3.50 2.92-4.20
36	4.21 2.66-5.46	4.21 2.67-5.85	4.06 3.48-4.56
PN	2.54 0.96-3.48	2.02 1.17-2.86	2.05 1.15-2.92

α_2 Globulin Fraction

Mean Values and Range (%)
(Number of estimations in brackets)

Weeks	I (16)	I+F (18)	Cu (4)
20	8.91 4.56-10.40	8.77 5.60-10.98	8.68 6.62-10.50
36	9.23 6.25-11.20	10.15 6.54-15.26	9.02 7.50-11.04
PN	7.41 4.40-10.50	7.97 5.67-9.73	6.49 5.26-8.64

β Globulin Fraction

Mean Values and Range (%)
(Number of estimations in brackets)

Weeks	I (16)	I+F (18)	Cu (4)
20	10.15 7-10-12.60	10.50 8.06-13.05	8.62 7.72-9.80
36	12.50 8.83-16.37	11.50 8.40-15.05	12.26 10.10-13.60
PN	8.16 5.38-11.40	8.10 5.84-10.50	7.21 5.80-8.40

 γ Globulin Fraction

Mean Values and Range. (%)
(Number of estimations in brackets)

Weeks	I (16)	I+F (18)	Cu (4)
20	11.63 9.30-16.8	11.17 7.60-14.40	12.98 9.90-16.80
36	11.23 8.10-17.25	10.89 8.63-13.76	11.54 7.94-15.35
PN	12.39 8.95-15.7	10.75 8.35-15.9	11.62 8.10-14.3

The differences were compared only in the I and I+F groups because of the small number of results available in the Cu group. The only significant differences found were in the post-natal specimens when the mean values of α_1 and γ Globulin were significantly higher in the group which had been on Iron than in the group which had been on Iron + Folic acid ($p < 0.02$)

Variations in the Protein Electrophoresis Patterns

The mean values of the protein fractions in the different groups, on the whole, follow the pattern usually described in pregnancy (Mack, 1955; Brown et al 1959).

Whiteside (1960) observed that in 50% of his cases, all treated with iron from early pregnancy, there was a significant increase in plasma volume from the first estimation to a second made between 28 and 36 weeks. This did not occur in the other 50% or in the red cell volume of either group. Protein electrophoresis showed a variation in the usual pregnancy pattern in the group with increased plasma volume. It is usually reported that albumin and γ globulin fall in pregnancy while α_1 , α_2 , and β globulin rise. In the cases with increased plasma volume Whiteside found that α_1 and α_2 globulin fell.

The results in the present study were inspected and it was found that there was marked individual variation of the pattern. Eight of the 16 women (50%) in the group on iron showed a fall of α_1 and/or α_2 globulin between the readings at 20 and 36 weeks. This pattern was present in 3 of the 18 (17%) women on iron + folic acid. Plasma and red cell volumes for these patients were extracted and compared with the volumes in women who exhibited the more usual protein electrophoresis pattern:-

Plasma Volume

Mean Values and Range (ml)
(number of estimations in brackets)

Weeks	Normal	Atypical
20	3625 (23) 2240-6550	3413 (11) 2420-4240
Max 28-36	3664 (23) 2470-5260	4441 (11) 2560-5860
28	3444 (22) 2160-5650	3745 (11) 2560-5860
36	3346 (21) 2160-5260	3936 (11) 1940-5430
40	2937 (12) 1910-5240	3126 (8) 2330-4660
PN	2330 (23) 1850-3560	2472 (11) 1770-3710

Red Cell Volume

Mean Values and Range (ml)
(Number of estimations in brackets)

Weeks	Normal	Atypical
20	1714 (23) 1000-2900	1564 (11) 1080-1970
Max 28-36	1778 (23) 1170-2790	2179 (11) 1340-3070
28	1635 (22) 900-2310	1767 (11) 1140-2940
36	1677 (21) 1120-2790	1961 (11) 1180-3070
40	1578 (12) 1120-3070	1686 (8) 1370-2260

Weeks	Normal	Atypical
PN	1271 (23) 850-1595	1312 (II) 910-1930

Neither plasma or red cell volume showed any significant differences when the comparisons were made between the mean values at 20, 28, 36 and 40 weeks or at the post-natal visit. However, when the highest value at either 28 or 36 weeks was taken it was found that this mean value was significantly higher than the mean value at 20 weeks in the atypical group. This applied to both plasma volume and red cell volume. Plasma volume, $p < 0.01$, and red cell volume, $p < 0.005$. In addition the comparison of mean maximum values (28-36 week) between the normal and atypical groups showed a higher level for the atypical group of borderline significance. Plasma volume $p < 0.05$ and red cell volume, $p < 0.05$.

Maternal Weight Gain. (Vol. 2. p. 105).

It was possible to estimate maternal weight gain in most of the cases as the stated pre-pregnancy weight was available. This was subtracted from the last weight in the ante-natal record if this was within two weeks of delivery. The weights of the child and the placenta were subtracted from this figure to give a reasonable approximation of weight gained by the woman during pregnancy. The mean values for the groups I, I+F and Cu were compared but no significant differences were found.

Baby Weights. (Kgms)

Group	Mean	Range	Standard Error
I (51)*	3.340	2.015-4.380	0.075
I+F (51)	3.500	2.709-4.824	0.078
Cu (16)	3.290	2.580-4.086	0.095

* The group on iron included two sets of twins.

The mean values for the three groups were compared but no significant differences were found.

Placental weights. (Kgms)

Group	Mean	Range	Standard Error
I (47)	0.691	0.430-0.990*	0.021
I+F (48)	0.694	0.200-1.150	0.037
Cu (14)	0.631	0.454-0.850	0.061

* Excluding twin placentae.

No significant differences were found between the mean values in the three groups studied.

Discussion

Expansion of the blood volume would be expected during pregnancy because of the increased vascular bed. Enlarged blood vessels are found, not only in the uterus and placenta, but also in the breasts and in dilated vessels distal to the large pregnant uterus. Rovinsky & Jaffin (1966) demonstrated that after 28 weeks the major part of the hypervolaemia was distributed to the maternal venous system and lower extremities since the central blood volume which represents the placenta and uterine vascular bed forms a progressively smaller proportion of the total blood volume as pregnancy continues. Size of the placental bed is, however, also related to the degree of hypervolaemia. Pritchard (1965 b) and Rovinsky and Jaffin (1965) report greater blood volume increments in twin pregnancies and the latter authors also found a positive correlation between plasma volume increment near term and placental mass.

An increased blood volume is also necessary as part of the adaptation needed to carry additional nutrients to the foetus and waste products, including heat, away. It is not surprising therefore that Hytten & Paintin (1963) find a correlation between the birth weight of the baby and maximum plasma increment. These mechanisms come into action surprisingly early in pregnancy. Cardiac output and renal flow are both increased by 10 weeks (Hamilton, 1949; Palmer & Walker, 1949; Sims and Kranz, 1958) and increased plasma volumes are apparent by the end of the first trimester (Hytten & Paintin, 1963).

There is a relationship between plasma volume and red cell volume

as is illustrated in cases of severe anaemia. In severe chronic anaemia there is usually a modest fall in total blood volume (McMichael et al, 1943) due to a marked fall in red cell volume which is largely compensated by an increased plasma volume (Gibson et al, 1939). A similar pattern is found in untreated pernicious anaemia (Gibson, 1939). A very recent publication of Harrison (1967) describes blood volume changes in severe anaemia of pregnancy. He found an increase in plasma volume greater than that shown by normal controls which served to maintain total blood volume within the normal range. In a few cases with haematocrit readings below 14% the plasma volume increase was not enough to replace the red cell deficiency and consequently total blood volume was reduced.

In addition it would seem that a degree of haemodilution is desirable in pregnancy as a protective mechanism to the heart (Hamilton 1950). It therefore seems possible that overstimulation of red cell production could be accompanied by a further increase of plasma volume to maintain optimum blood viscosity. It has not, however, been demonstrated that red cell mass in excess of normal requirements can be produced in pregnancy. The work of Dagher et al (1965) although done mainly on men would suggest otherwise. These authors consider that red cell volume reflects body cell mass and would appear to be 'tailored' to match the size of the body cell mass to which the erythrocytes must transport oxygen.

Red cell volume would be expected to increase in a normal pregnant woman in proportion to the increased tissue mass requiring oxygen but expansion of the plasma volume provides the main component of the

hypervolaemia. The reasons for this increase would seem to be largely mechanical although the precise mechanism is not clear. Neuroreceptors which could mediate such a change have not been found (Vorys et al, 1963). Other factors have been considered. Aldosterone levels are raised in pregnancy and hypervolaemia has been shown to accompany primary hyperaldosteronism (Biglier and Forsham, 1961). Pritchard (1965b) suggested that the growth hormone-like substance in the placenta described by Josimovitch & Atwood (1964) may contribute to the hypervolaemia. High concentrations of oestrogen and progestogen are present in pregnancy. Witten & Bradbury (1951) found that granulosa cell tumours in mice were associated with hypervolaemia. Pritchard (1965) referring to his own unpublished work mentions that the blood volume can be increased in normal adults when large doses of oestrogen are given. The increases however, were not nearly as great as those which occur in pregnancy. The presence of a foetus is not essential for the development of hypervolaemia as Pritchard (1965a) reported increased blood volume of as much as 50% over the non pregnant state in some but not all cases of hydatidiform mole.

One of the earliest attempts to measure the increase in blood volume in human pregnancy was by Miller, Keith and Rowntree in 1915. It was not until 1934 that a systematic serial study was made by Dieckmann & Wegner using Congo Red. This dye had disadvantages and modern work really began with the study of Thomson et al in 1938 in which they used Evans blue dye as a tracer. Since that time many studies have been made.; In some series plasma volume has been estimated and other values

calculated, making use of haematocrit readings. Since the introduction of radioactive tracers it has been possible to make direct estimations of red cell volume. The most prominent feature of the published papers on the subject is the marked variability of results. The individual range at any week of pregnancy studied is very wide. Lowenstein et al (1950) found the change in plasma volume to range from - 4% to + 77% on the non pregnant value and Lund (1951) + 14% to + 121%. Similar variations are found in the work on red cell volumes. The average maximum increase ranges from 110 ml (Roscoe and Donaldson, 1946) to 560 ml (Werkö et al, 1948). The most carefully planned recent work on the subject is from Aberdeen and consists of the publications of Hytten & Paintin (1963) on plasma volume and Paintin (1962) on red cell volume. These are summarised by Drs. Hytten and Leitch on p. 24 of their publication "The Physiology of Human Pregnancy" and are the figures used for comparison with those found in the present investigations.

The results of the present work are illustrated by scattergrams showing the individual readings for blood volume, plasma volume, red cell volume and haemoglobin mass (Figs. 15, 16, 17 and 18). On inspection the most striking feature is the wide range of individual readings at the different weeks of pregnancy. This is not so noticeable when the post-natal readings are studied and there is also a much closer grouping in women on iron + folic acid. In the non-pregnant state there is close correlation between plasma volume and body weight and height, Hytten & Paintin (1963). It is possible that during pregnancy there is individual variation of uterine size and dilatation of maternal blood vessels which would explain this wide range of results.

Many previous workers have expressed their results as percentage

increase on non-pregnant values. For this purpose they have used either post-natal values in the same women or non-pregnant controls. Hytten & Paintin (1963) have shown that this method is fallacious as the increments are a function of the non-pregnant values which will vary with the size of the woman. The volume of the increment will also depend to some extent on the size of the child. In this investigation it was thought that post-natal values in the different groups might vary depending on the treatment given during pregnancy. For this reason the patterns of volume behaviour in the different groups were, once again, to show the percentage variation on the 20 week levels (Figs. 19, 20.)

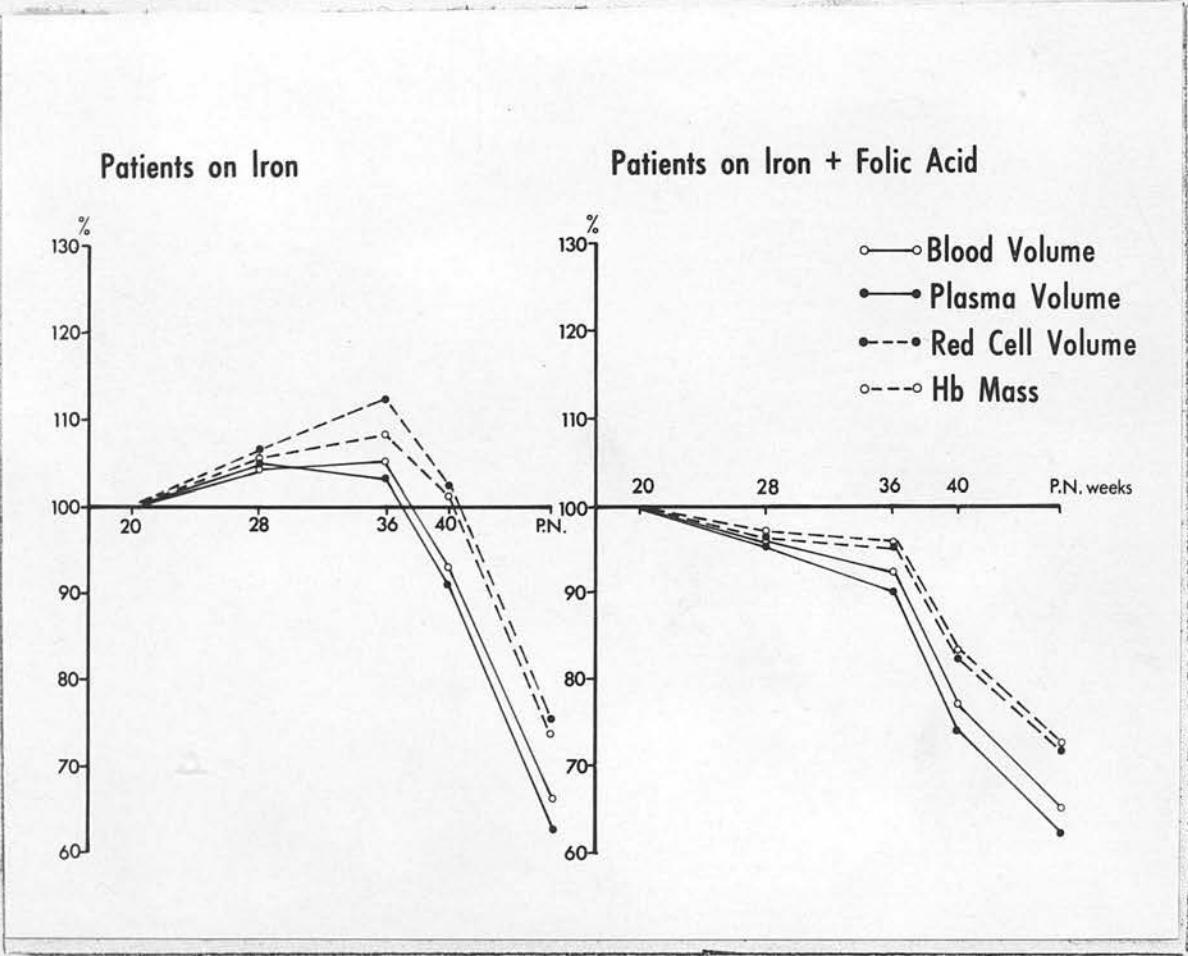
Post natal values in the present study are lower than those given by Hytten & Leitch. The mean values for all groups are compared below with the figures given by these authors and also with the post-natal volumes reported by Harrison (1966) in normal Nigerian women:-

	E.B.B.	F.E.H. & I.L.	K.A.H.
B.V.	3600	4000	3592
P.V.	2340	2600	2405
R.C.V.	1260	1400	1187

Red cell volume is 35% of the total volume in the present series and in the Scottish figures but is slightly less (33%) in Nigerian women. The lower volumes shown probably reflect the smaller average size of Welsh women as compared with Scottish women.

There was considerable individual variation of the stage of pregnancy at which the maximum volume increments were recorded. This makes the time of maximum increment as shown by the mean values of the

Fig. 19. Pattern of Volume Changes



groups studied of doubtful value. The group on iron showed a maximum increment for blood volume and red cell volume at 36 weeks and for plasma volume at 28 weeks. The maximum increment was at 20 weeks for all volumes in the I+F group and at 28 weeks in the Cu group. None of the groups showed a continued rise of red cell volume to 40 weeks as shown by Hytten & Leitch (1964). This might be due to the smaller number of readings available by this stage of pregnancy.

The diagrammatic patterns of volume behaviour shown in figures 19 and 20 have been drawn so that the relative behaviour of blood volume, plasma volume, red cell volume and haemoglobin mass can be studied together in each of the four groups (I, I+F, Cu & Cw.)

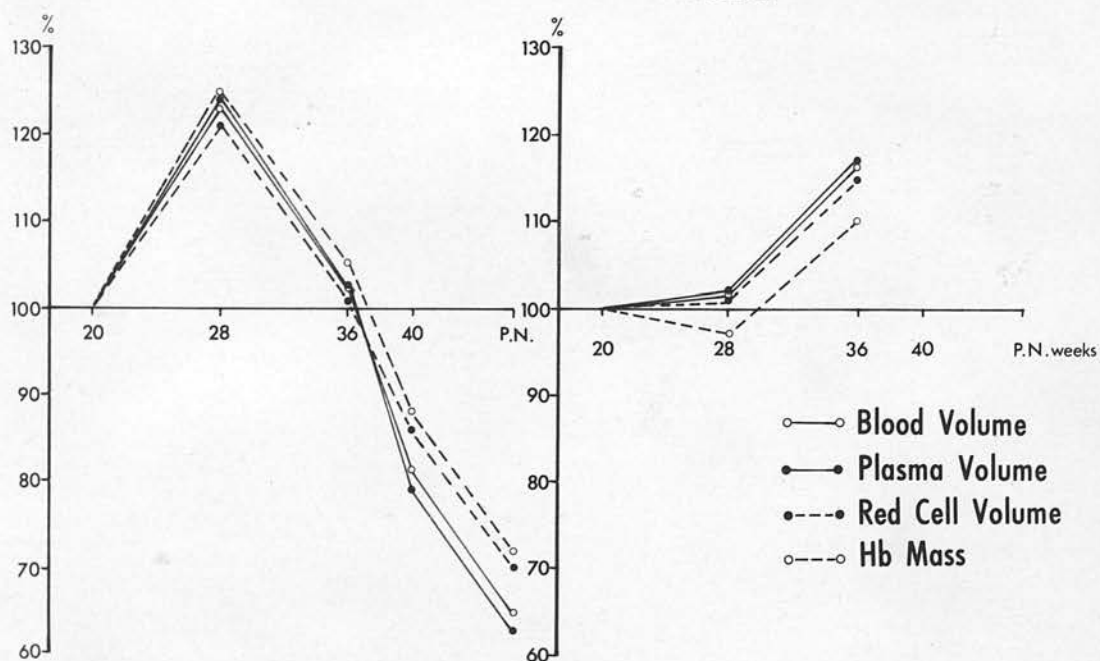
The group on iron show a pattern of volume changes similar to that usually reported. A large part of the volume increase has already occurred by 20 weeks and there is a continued slight rise to 36 weeks. The fall at 40 weeks is rather greater than is shown by Hytten and Leitch. Points of interest are the relative fall in plasma volume as the red cell volume reaches its maximum at 36 weeks and also the 'lag' in the increase of haemoglobin mass at this time compared with the red cell volume. One would have expected a comparable increase as well haemoglobinated cells should have been produced in women on an iron supplement.

The most interesting pattern is shown by the group on iron + folic acid. Maximum increments are reached at 20 weeks and fall through the rest of pregnancy. The fall mainly occurs in the plasma volume. Red cell volume

Fig. 20. Pattern of Volume Changes

Controls Completely Untreated

Controls which required Treatment while Untreated



and haemoglobin mass are very comparable and are reduced only 5% by 36 weeks while plasma volume is reduced by 11%. When Fisher's test for combining significance levels from different samples was applied to plasma volume it was shown to be significant at the 5% level. In addition on inspection of the individual results 15 of the 24 women in the I+F group (62%) showed this pattern of a fall in plasma volume as compared with 7 out of 20 women in the group on iron (35%) and 8 of the 25 women in the combined untreated groups (32%).

This variation in the usually reported behaviour of blood volume in pregnancy is difficult to explain. The Nigerian women studied by Harrison (1966) were also taking iron and folic acid and did not behave in this way.

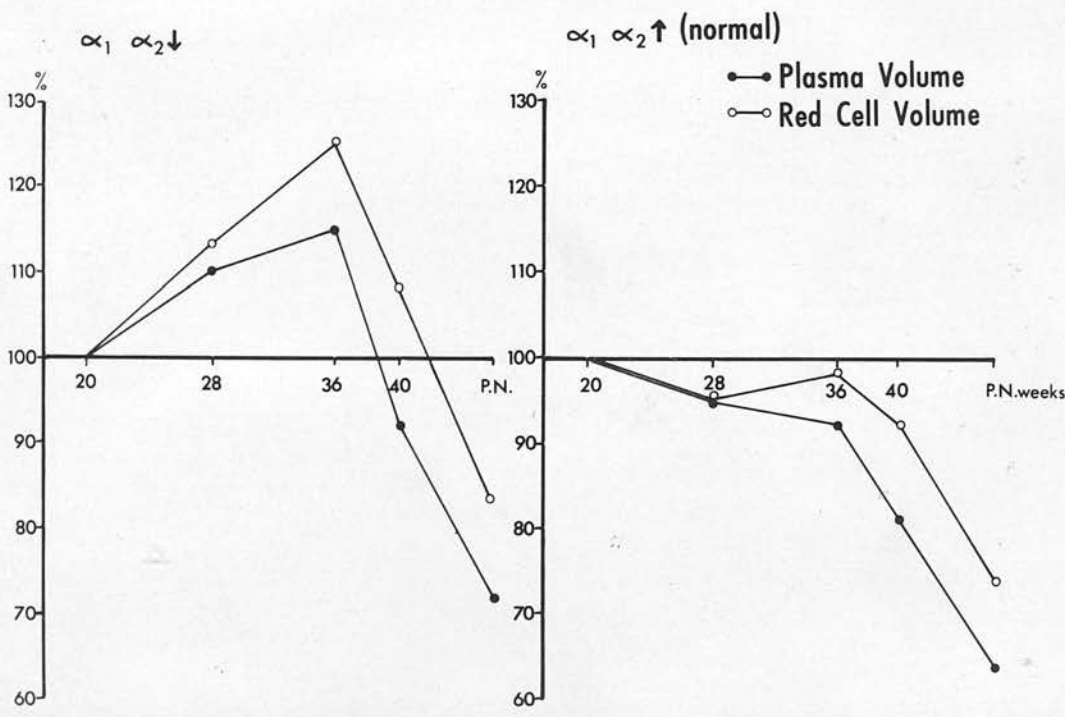
Additional possible differences between these women and those of other groups were investigated. The case records of all of the women in the project were studied and it was found that slight to moderate oedema was present in the following proportions of women in the different groups:-

Group	Total	No. with oedema	%
I	49	14	29
I+F	51	27	53
Cu	16	3	19
Cw	38	18	47

It is suggested that the high incidence of oedema in the Cw group is associated with anaemia. The unexpected finding is that almost twice as many women in the group on iron + folic acid showed some degree of oedema as in the group on iron only. The maternal weight gain was compared and no significant differences were found. Maternal weights and presence or absence

Fig. 21. Pattern of Volume Changes

Red Cell Volume and Plasma Volume Related to behaviour of α_1 and α_2 Globulin
20 week Values = 100%



of oedema were both taken from the clinical records retrospectively. In any future work accurate measurements would be necessary. These rather rough findings suggest the possibility that there is some relationship between folic acid metabolism and intra-vascular and extra-vascular fluid balance. In cases where serum folate levels were available mean values for the pregnancy were compared in women with and without oedema. No significant differences were found.

One further piece of evidence suggests the possibility of a relationship between folic acid metabolism, plasma globulins and plasma volume. Whiteside (1960) found that increased plasma volumes were associated with a fall in α_1 and α_2 globulins (these usually rise in pregnancy). To investigate this further protein electrophoresis patterns were studied. Eight of the ¹⁶women (50%) with results available in the group on iron showed a fall in α_1 and / or α_2 globulin. This is the same incidence as reported by Whiteside. His patients were also taking iron. Eighteen women in the I+F group had results available and only 3 of these (17%) showed a fall in α globulins. Plasma and red cell volumes for women with atypical and normal globulin patterns were extracted and a diagrammatic representation of the mean values is shown in Figure 21. It will be seen that women with normal globulin behaviour show plasma and red cell volume patterns very similar to those demonstrated in Figure 19 for women on iron + folic acid.

A simpler explanation is perhaps that of Dr. Victor Herbert (personal communication). He suggested that the behaviour of the plasma

volume in the I+F group reflected the correction of a latent folic acid deficiency which remained uncorrected in the other groups. As has already been mentioned in Chapter 3 a proportion of the women in all groups entered the investigation at 16 weeks. The mean plasma volume for the 15 women who began to take iron + folic acid at 16 weeks is 3535 ml while in the eight women who did not start until after the first plasma volume estimation at 20 weeks it is 4456. Similarly in the group on iron when divided in this way the mean values are 3478 ml and 4036 ml respectively. In this group 10 of the 20 women began to take iron at 16 weeks. The numbers of patients involved here are small but the figures provide some support for Dr. Herberts view that plasma volume can reflect even latent anaemia.

The patterns of volume changes in the untreated groups have now to be considered. The main feature of interest in the pattern shown by the Cu group is the close relationship between red cell volume and plasma volume. In addition the relative increase in haemoglobin mass does not fall below the increase for red cell volume. The peak at 28 weeks is pronounced but is due to two high readings which, although within two standard errors of the mean have undue weight in the small number of results available in this group. It should also be noted that the mean values at the post-natal visit in the Cu group are lower than in the treated groups. Plasma volume is 159 ml lower than in the I+F group and 137 ml lower than in the group on iron. The differences in red cell volume are 136 and 120 ml and in haemoglobin mass 49 and 53 grammes. The proportion of red cell volume in the total blood volume is 34% as compared with 35% in the treated

groups. These differences are not significant but suggest that even apparently normal women who showed no evidence of anaemia during pregnancy are at a slight disadvantage after pregnancy when compared with women who received supplements.

Haemoglobin mass in the Cw group shows a different relationship to red cell volume than has been seen in the other groups. It is relatively much lower and probably reflects increasing hypochromia of red cells. The increase in red cell volume at 36 weeks in this group is difficult to explain. The mean values are, however, derived from only five readings.

In general, the range of individual readings is wide with a high standard error of the mean in most instances. It is felt, therefore, particularly where few readings are available, that the main value of these estimations is in the relationship of red cell volume, haemoglobin mass and plasma volume in their respective groups rather than in the comparison of mean values between the groups.

Chapter 6.

Synthesis and Conclusions

The information gained in this investigation can be considered under two headings:-

- 1) The effect of iron and folic acid supplements on erythropoeisis and plasma volume.
- 2) The relationship between iron, folic acid and vitamin B₁₂.

1. The Effect of Supplements of Erythropoeisis and Plasma Volume

A normal woman with adequate iron stores, normal absorption and on a good diet would seem to undergo physiological changes during pregnancy designed to increase erythropoeisis. Iron stores are utilised and the plasma transferrin pool is increased and becomes more active. These changes result in better absorption of iron. Increased quantities of folic acid are also needed and become available perhaps from increased absorption and possibly from utilisation of red cell folate (Grzesiukowicz et al, 1965). The body normally contains large stores of vitamin B₁₂ and the rate of absorption would seem to be adequate even with the additional burden of pregnancy. (Heyssel et al, 1966).

Under these conditions red cell mass is able to increase to the optimum needed for oxygenation of additional tissues. At the same time, there is an increase in plasma volume of sufficient amount to supply the increased vascular bed, reduce blood viscosity and also nourish and remove waste products from the foetus.

Unfortunately rather less than 30% of pregnant women seem to

behave in this way. Seventy to eighty per cent of pregnant women present clinically with haemoglobin levels below 12 g/100 ml. It is routine in most clinics to give such women iron and in some centres folic acid as well. Such women are not necessarily "anaemic" as their red cell volume can be within normal limits. The fall in haemoglobin concentration is then due to excessive increase of plasma volume. Although in some women increased plasma volumes are difficult to explain, the findings in this investigation suggest that it can be associated with iron and/or folic acid deficiency even if this is not sufficiently severe to depress production of red cells. The red cells produced, however, may contain less than the optimum amount of haemoglobin.

Erythropoiesis is stimulated by therapeutic doses of iron in this series as was reported by Paintin et al (1966). This is reflected by red cell volumes which are in the region of 200 ml higher than in normal untreated women (excluding the two exceptionally high readings in the Cu group at 28 weeks). In the same way, plasma volumes in women on iron become progressively greater than the plasma volumes of untreated normal women as pregnancy continues. It is suggested that this is a response to the increased red cell volume and occurs to preserve the optimum degree of haemodilution and thence blood viscosity.

It is also possible that excessive iron causes a relative deficiency of folic acid and that this is reflected by increased plasma volume. There are some indications in the present work that this may be mediated by alterations in plasma globulin patterns. Further work is required here using

more sophisticated methods to study protein fractions and also in women with folic acid deficiency anaemia of pregnancy.

When folic acid is given in addition to iron the mean red cell and plasma volumes are more comparable to those recorded for normal untreated women. It would seem that in some unexplained way the metabolism of iron and folic acid are related so that when both are present in comparable amounts erythropoiesis is no longer stimulated beyond the requirements of the body cell mass.

2. The relationship between Iron, Folic Acid and Vitamin B₁₂

In pregnancy there is an increased need for iron, folic acid and vitamin B₁₂, both to increase the red cell mass of the mother and to supply the foetus. Serum iron and serum folate levels vary in relation to food intake and so only given an approximate guide to status in the patient. Total iron binding capacity and % saturation ^{of} transferrin provide a more reliable index of iron status. Recent work (Lawrence, 1967; personal communication) suggests that red cell folate levels would have been of more value in the case of folic acid but these are not available.

Normal untreated women in the present investigation show increased activity of the transferrin pool as reflected by raised mean values of total iron binding capacity and a fall in % saturation. Serum iron levels also fall. It is thought that these changes are an indication of the physiological adaptation necessary to promote increased absorption and utilisation of iron. Mean serum folate levels remain reasonably uniform throughout pregnancy and show little change from non pregnant levels in the same patients. Mean serum B₁₂

shows a moderate fall. It is possible that this reflects a physiological adaptation due to pregnancy but the reasons for the change and the mechanism cannot be explained.

The addition of therapeutic quantities of iron produce the expected increase in serum iron levels but there seems to be a depression of activity of the transferrin pool, as, by the end of pregnancy, mean total iron binding capacity falls and there is an increase in mean % saturation. There is a rather greater fall in mean serum B₁₂ levels and serum folate levels fall slightly throughout pregnancy.

When folic acid is also given raised serum iron levels are still found but there seems to be recovery of activity of the transferrin pool. This is shown by the continued increase of mean total iron binding capacity throughout pregnancy which is associated with lower mean % saturation levels than are found in the group on iron. This again suggests that iron and folic acid should be present in comparable amounts for optimal utilisation of both. Reports of the development of megaloblastosis and other evidence of folic acid deficiency, in association with severe iron deficiency, support this thesis. The fall in serum folate levels in the presence of excessive iron would also seem to indicate an association between iron and folic acid metabolism.

In the presence of additional iron and folic acid, mean serum B₁₂ levels show a greater initial rate of fall and a slightly greater number of low individual readings are recorded during the pregnancy. The lowest mean value, however, is comparable with that seen when iron alone is given.

It is thought that these slight variations in behaviour of serum B₁₂ levels in the treated groups also indicate that vitamin B₁₂ metabolism is closely associated with iron and folate metabolism and that once again there is a relative deficiency in one variable as compared with an excess of one or both of the others. It is suggested that the changes are less pronounced because of the large stores of vitamin B₁₂ normally present in the body.

Changes in the mean lobe counts in the treated groups would seem to suggest that both iron and folic acid have a quantitative effect in increasing the rate of maturation in marrow cells. There was little change in the mean lobe counts through pregnancy in the normal untreated group. The mean value at 20 weeks was, however, significantly lower in the Cu group. This may be an indication of initial normality when compared to the treated groups. The latter probably included some women with latent deficiency of iron and/or folic acid.

Conclusions

Iron and folic acid nutrition would seem to be optimal in not more than 30% of pregnant women. The modern custom of giving iron supplements would be improved by giving folic acid as well to ensure a better metabolic balance. The combination is beneficial in the majority of women and was not found to be disadvantageous in women who did not need supplements.

Examination of the haemoglobin concentration is usually the only test available in a busy ante-natal clinic to distinguish the small proportion of women who do not need supplements from those who would benefit. There is no clear distinction between the haemoglobin levels in the two groups by the 20th week of pregnancy, but results available earlier in pregnancy suggest

that haemoglobin concentrations of less than 13.0 g/100 ml before 16 weeks have a close association with latent deficiencies.

It is probable that smaller doses of iron and folic acid have been used in this investigation would be sufficient in most women.

In the case of folic acid 300 µg/day would be, a safer quantity because of the remote possibility of significant vitamin B₁₂ deficiency.

These observations are not intended to apply to women who are anaemic in early pregnancy or who become anaemic by reason of metabolic defects or defective absorption.

Routine supplements of iron and folic acid during pregnancy would be of some assistance in protecting women from the minor ailments so often associated with chronic anaemia in the later child-bearing years.

S U M M A R Y

1. Reproduction is a physiological function of the body and one would expect normal women without intercurrent disease to remain in good health during pregnancy and after delivery. Minor ill health during and after child-bearing is common in women until the time of the menopause. This is frequently related to chronic anaemia. As a result of such observations it is now common practise to give iron, and in some centres folic acid also, as a routine supplement to all pregnant women.
2. A proportion of women will be given supplements unnecessarily or in excess of their requirements. It is desirable to know what proportion of women do not need additional iron and folic acid and also whether it is possible to recognise these women in early pregnancy. It would also be of value to know whether women in general benefit as shown by tests made to demonstrate the status of iron, folic acid and vitamin B₁₂ nutrition or whether mineral and vitamin supplements in excess can cause unexpected metabolic problems.
3. In this investigation comparisons were made between untreated women, women on 194 mg. ferrous sulphate daily and women on 194 mg. ferrous sulphate + 3.4 mg. folic acid daily. Two hundred women, 20 weeks pregnant or less entered the investigation and 154 were followed through to the post-natal visit 6-8 weeks after delivery. Women were placed in the treatment groups by means of randomised lists stratified by age, parity and initial haemoglobin level. Only women with haemoglobin levels of more than 10.0g/100 ml at 20 weeks were admitted to the investigation. In retrospect this was probably too low and a lower limit of 11.0 g/100 ml would have been preferable. In the same way

although the range of age and parity was comparable in the three groups, in a physiological study of this sort it would probably have been better to have studied women in a smaller age range and of uniform parity.

4. For the purpose of this communication, results at 20, 28, 36 and 40 weeks are used together with the results at the post-natal visit.

Haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, serum iron, total iron binding capacity, latent iron binding capacity and % saturation estimations are available on all women. Blood films were also examined in all cases and mean lobe counts made. Serum B₁₂ and serum folate estimations are available on half of the women in each treatment group and blood volume, plasma volume, red cell volume and haemoglobin mass estimations in the other half. The lists for these sub-groups were also randomised.

5. The results are analysed and discussed in relation to the findings reported by previous authors.

6. The information gained from the various investigations and from relevant publications of previous observers is finally synthesised under two headings:-

- a) The effect of iron and folic acid supplements on erythropoiesis and plasma volume.
- b) The relationship between iron, folic acid and vitamin B₁₂ metabolism.

7. It is concluded that:-

- a) Iron and folic acid nutrition are optimal in not more than 30% of women.

- b) It is not possible to make a certain identification of all women who will benefit from treatment at the 20th week of pregnancy but there is some indication that haemoglobin levels of less than 13.0 g/100 ml before 16 weeks should be suspect.
- c) The metabolic balance is closer to the normal pattern when folic acid is given in addition to iron.
- d) There was no evidence to suggest that iron and folic acid had a disadvantageous effect when given unnecessarily.
- e) The observations apply to women with sub-clinical deficiencies. It is thought that supplements in these women would help in the prevention of mild chronic ill health in the later years of their child bearing career.
- f) It is suggested that smaller supplements than are given at present would be sufficient in most women. This applies particularly to folic acid and 300 µg/day would seem to be an optimal dose.

8. These conclusions are reached on information available at present but a number of matters require further elucidation

- a) It would be useful to repeat this study using smaller quantities of iron and folic acid and also to investigate the effect of a small vitamin B₁₂ supplement.
- b) There was evidence of a relationship between folic acid, plasma volume, serum globulins and intra and extra-vascular fluid balance. Further work with careful assessment of oedema and maternal weight gain would be of value. A more advanced study of

protein globulin fractions is also necessary.

- c) Folic acid seemed to modify the stimulation of erythropoeisis caused by iron. This needs to be repeated and if confirmed opens a further field for investigation in a study of the complex relationship between iron and folic acid metabolism.

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